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EDITED BY

SIMON FLEXNER, M.D.

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Herbert Douglas Taylor

Born 1889 Died 1918

At the meeting of the Board of Scientific Directors of The Rockefeller Institute for Medical Research, held on October 19th, 1918, the following note was placed on the Minutes:

"The Board of Directors of The Rockefeller Institute records with sorrow the death, on October 7th, 1918, in the service of his country, of Lieutenant Herbert Douglas Taylor of the Medical Corps of the United States Army. His notable contributions to science, his unswerving devotion to his chosen service for the general welfare, his inspiring idealism, and his genial comradeship won for him, in no common measure, the esteem, friendship, and love of his fellow workers of the Institute."

Lieutenant Taylor's short career in scientific research was marked by such energy, enthusiasm, and clearness of vision as were a real inspiration to his fellow workers. During his three and one-half years at The Rockefeller Institute his studies dealt mainly with problems associated with cancer and tuberculosis. Soon after the United States entered the war he offered his services and was appointed First Lieutenant in the Medical Corps of the Army, and assigned as instructor of medical officers in the War Demonstration Hospital and in the Laboratory Courses of the Institute. He threw himself into the new work with characteristic energy. Not content with teaching and the routine work of the War Demonstration Hospital, he started extensive investigations of the action of antiseptics on infected wounds and contributed important papers on this subject. At the beginning of the epidemic of influenza he took up certain phases of this problem to which he gave himself without reserve. In the course of his studies he contracted influenza and died after a short illness.

This number of The Journal of Experimental Medicine, contributed by the Department of which Lieutenant Taylor was a member, was prepared with his enthusiastic cooperation. It contains much of his own completed work. His fellow workers offer it as a memorial to a spirit and an example that will not die.

All the studies in this issue have been carried out in the section of the Division of Pathology and Bacteriology of which Dr. James B. Murphy has charge.

EFFECT OF DRY HEAT ON THE BLOOD COUNT IN ANIMALS.

III. Studies on Lymphoid Activity.

By JAMES B. MURPHY, M.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 1 to 3.

(Received for publication, October 17, 1918.)

The study of the function of the lymphoid tissue has, in the past, been productive of few leads of importance, due undoubtedly to the lack of methods for approaching the subject. It has not been possible, owing to the wide distribution of the lymphoid tissue throughout the body, to employ the method so productive in the study of deficiency of function in the glands of internal secretion brought about by partial or complete removal. Extensive investigations on animals and man after removal of the spleen, the chief lymphoid depot, has added little to our understanding of the function of this type of tissue. The loss under these conditions is so rapidly compensated for by hyperplasia of the lymphoid tissues that a diminished function cannot be detected. By utilizing the well known fact that lymphoid tissue is extremely sensitive to x-rays, we were able to develop a method by which practically the entire lymphoid tissue of the body can be destroyed with a minimum destruction of other tissue and slight, if any, effect on the general health of the animal.[1] The method consisted of small, repeated doses of x-rays distributed over from 7 to 21 days, depending on the size and resistance of the animal. The experiments carried out on the delymphocytized animals led to the following results: (1) destruction of the mechanism of resistance against implants of foreign tissue;[1] (2) lowered resistance to inoculated cancer grafts;[2] (3) destruction of established immunity to cancer;[3]

[1] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1. Mottram, J. C., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917–18, xc, 1.

(4) lowered resistance to tuberculosis in mice,[4] guinea pigs,[5] and monkeys;[6] (5) lowered resistance to poliomyelitis in monkeys.[7]

From the experimental side we have been able to get suggestive results after the stimulation or apparent increased activity of the lymphoid tissue. The chick embryo, normally with no resistance against transplants of heteroplastic tissue, can be rendered resistant by suitable transplants of adult lymphoid tissue.[8] A single small dose of x-rays, sufficient to stimulate somewhat the circulating lymphocytes, increased the resistance of mice to autotransplants of spontaneous cancers.[9] Mice with high lymphoid counts resulting from splenectomy or from the reaction engendered in a cancer-immune animal after inoculation with a cancer graft show a marked increase in resistance to bovine tuberculosis.[4,10] All the results given above suggest, rather than prove, that the lymphocyte is an active agent in these varied processes. It seems necessary to test more completely the effect of stimulation by other means.

Many methods have been tested to produce an extensive and enduring increase in the lymphocyte and lymphoid tissue. Among these were the use of a number of drugs and dyes, ultra-violet light, and sunlight.[11] Except in the cases of the two latter agents, the results were unsatisfactory. Either the increase was too slight to be of experimental value, or not of long enough duration, or the incidental disturbance to the general health of the animal was too great. One highly promising method has been found; namely, the use of intense dry heat.

Method.

The source of the heat in these experiments was an electric bulb of frosted glass, giving the maximum heat for the amount of light generated. The method of application varied with the size of the

[4] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[5] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.
[6] Taylor, H. D., unpublished observation.
[7] Amoss, H. L., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 115.
[8] Murphy, Jas. B., *J. Exp. Med.*, 1914, xix, 513.
[9] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.
[10] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.
[11] Taylor, H. D., *J. Exp. Med.*, 1919, xxix, 41.

animal and its ability to stand the higher temperatures. For mice an ordinary iron tripod 9 inches high and $5\frac{1}{2}$ inches at the base was covered on the sides with cardboard. A space was left at the bottom for free circulation of air. This was placed over an electric "Sunbeam" heating lamp of 110 volts, 80 watts direct current. A small circular cage of the same size as the opening was placed on top of the tripod. This was made of galvanized iron with a wire mesh bottom ($\frac{1}{4}$ inch mesh). The distance from the top of the lamp to the bottom of the cage was 3 inches. A thermometer was introduced parallel to and about $\frac{1}{4}$ inch below the bottom of the cage, directly over the light. After the light was turned on, the temperature was allowed to rise to 55°C. before the mouse was put into the cage. The animal was allowed to remain 5 minutes, this being about the time required for the temperature to rise from 55–65°C. If the temperature rose too rapidly it was found advisable to shut off the light until the thermometer registered between 57° and 59°C. and then allow it to increase again to 65°. For mice the temperature should never exceed 65°, as they can survive only 1 or 2 minutes at a higher temperature than this. A small piece of blotting paper was placed in the bottom of the cage just large enough for the mouse to rest on. In our experiments the loss was less than $\frac{1}{2}$ of 1 per cent, and only occasionally were burns encountered.

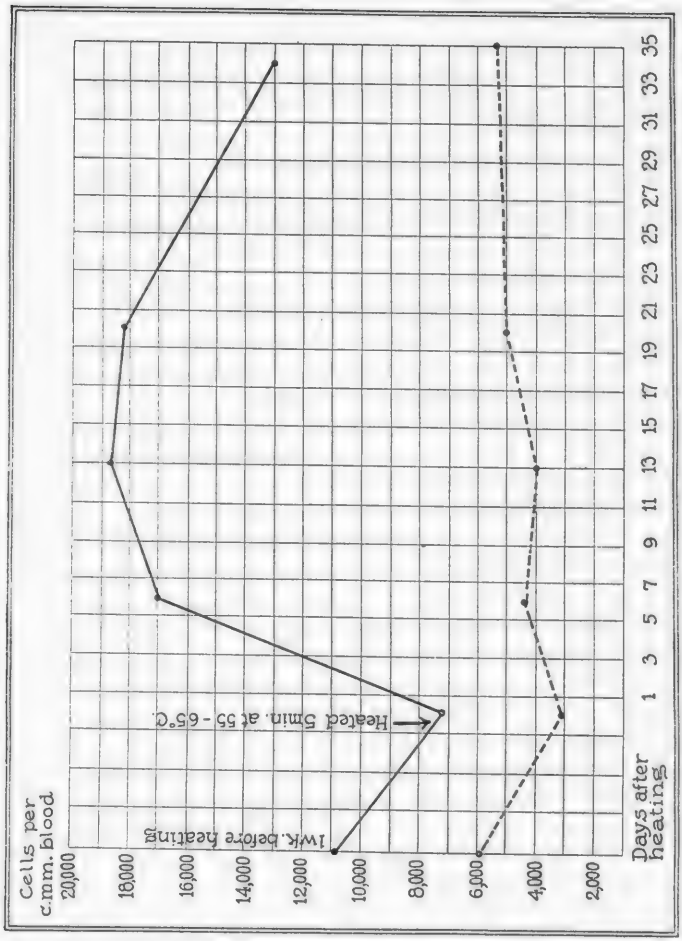
For heating larger animals, such as rats or guinea pigs, a wooden box 9 by 6 inches and $4\frac{1}{2}$ inches high was used with an electric carbon heating lamp 25 volts, 100 watts direct current, suspended directly over it. This lamp was surrounded by a metal reflecting cup. The distance from the lamp to the top of the box was 10 inches. The thermometer was placed at the level of the body of the animal. Rats and guinea pigs were found much more susceptible to higher temperatures than mice. A temperature of 60°C. or more caused extreme discomfort. The animals can, however, stand a more prolonged exposure at the lower temperature than can mice. For this reason it was found advisable to reduce the maximum temperature and increase the time of exposure for these animals.

Effect of Dry Heat on the Leucocytes in Mice.

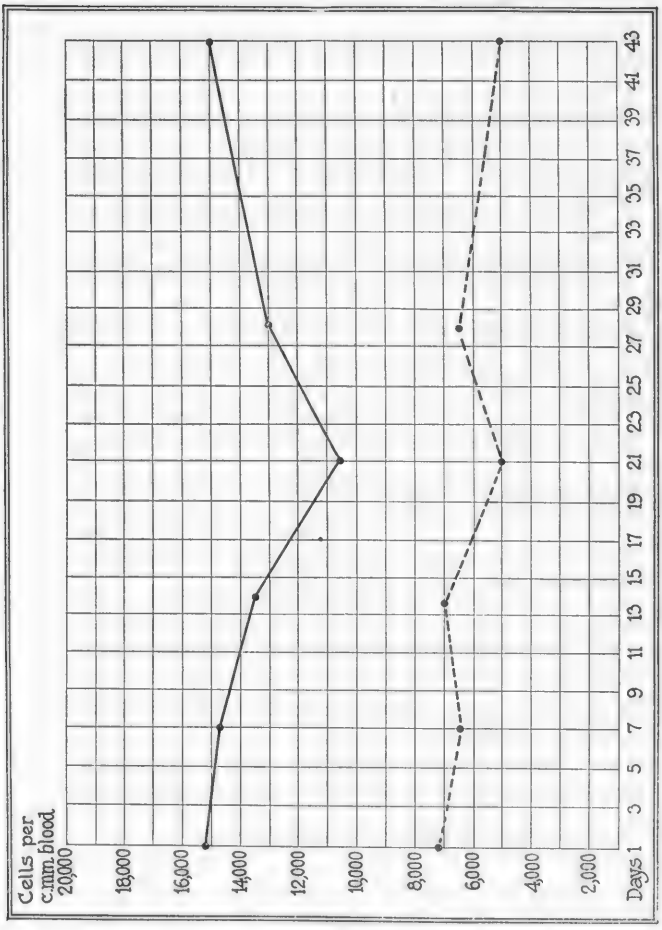
Experiment 1.—Total and differential leucocyte counts were made on fifteen healthy mice of about the same age and size. 1 week later ten of these animals were heated over an electric lamp at a temperature ranging from 55–65°C. for 5 minutes. White cell and differential counts were made immediately after this procedure, and counts were also made on five remaining animals as controls. All the fifteen animals were counted again, 7, 14, and 21 days after heating. On charting the results of the counts it was found that immediately after heat exposure there was a uniform decrease in both principal types of white cells, the lymphocytes and the polymorphonuclear leucocytes, which averaged about 3,000 cells per c.mm. of blood. The count on the five unheated mice showed no appreciable change. 7 days after heating it was found that there was an increase of from 5,000 to 10,000 cells per c.mm., this increase being almost entirely in the lymphoid group. The polymorphonuclear leucocytes remained lower than they were in the original count. The blood picture in the five control mice was similar to that at the previous determinations. 14 days after heating, the lymphocytes had increased to a figure above the original count in some of the heated animals to the extent of from 12,000 to 14,000 cells per c.mm. of blood, while the polymorphonuclear leucocytes still remained below the original determination in practically all the animals. The control animals showed no change beyond that well within the bounds of experimental error. 21 days after heating, the lymphocytes, although several thousand cells above the original count, had fallen off somewhat from that of the previous week. The polymorphonuclear cells at this point showed a tendency to rise. These results are graphically recorded in Text-fig. 1 for the heated animals and Text-fig. 2 for the controls.[12]

Experiment 2.—White blood counts were made on ten healthy mice of approximately the same age and size. A week later five of these (Group I) were heated for 5 minutes at a temperature of from 55–65°C. Immediately afterward blood counts were made on these and the five unheated animals (Group II). A week later the control mice (Group II) were heated in the same manner as that employed with Group I the previous week. Counts were made on both groups at this period and repeated 7 and 21 days later. Group I showed an average decrease of 2,500 lymphocytes per c.mm. of blood after heating, while the polymorphonuclear leucocytes dropped more than 6,000 cells. Group II, which was used as the control up to this point, showed little change in the blood picture. Counts a week later on Group I, 1 week after heating, showed an average increase of over 6,000 lymphocytes per c.mm. above the normal level. The polymorphonuclear leucocytes had recovered somewhat from the effect of the heat, but were still below the normal. Group II at this time, after having been counted twice with

[12] From past experience it was found that if counts were made more often than once a week it caused too great a fluctuation in the count.

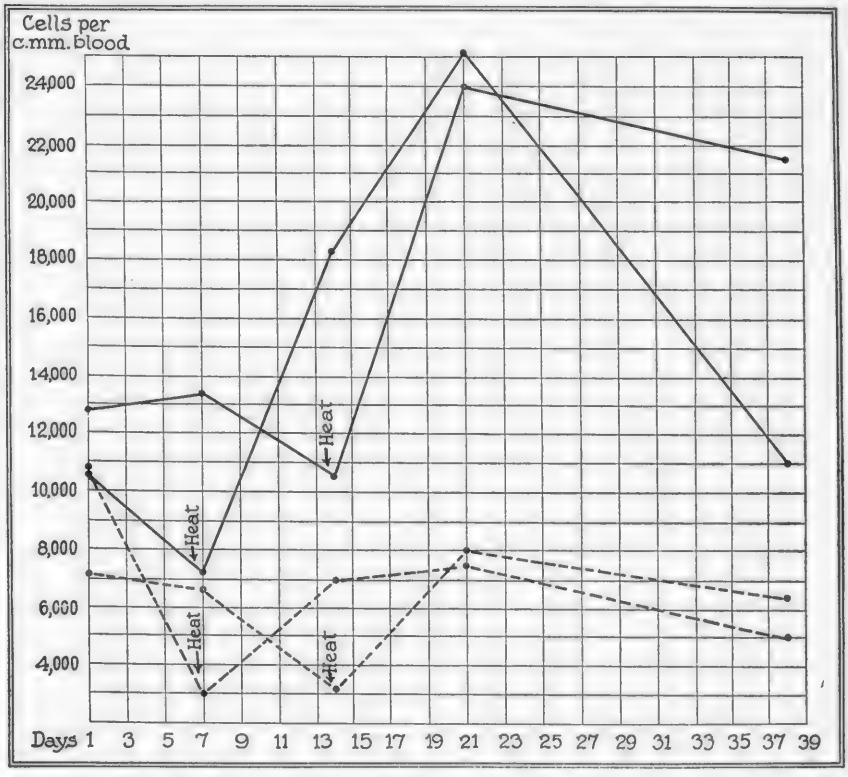


TEXT-FIG. 1. Composite curve of the white blood cell counts on ten mice before and after exposure to dry heat. ——Lymphocytes. .....Polymorphonuclear leucocytes.



TEXT-FIG. 2. Composite curve of the white blood cell counts on five normal mice of the same strain as those in Text-fig. 1. The animals were kept under the same conditions, and counts were made at the same time. ———Lymphocytes. .....Polymorphonuclear leucocytes.

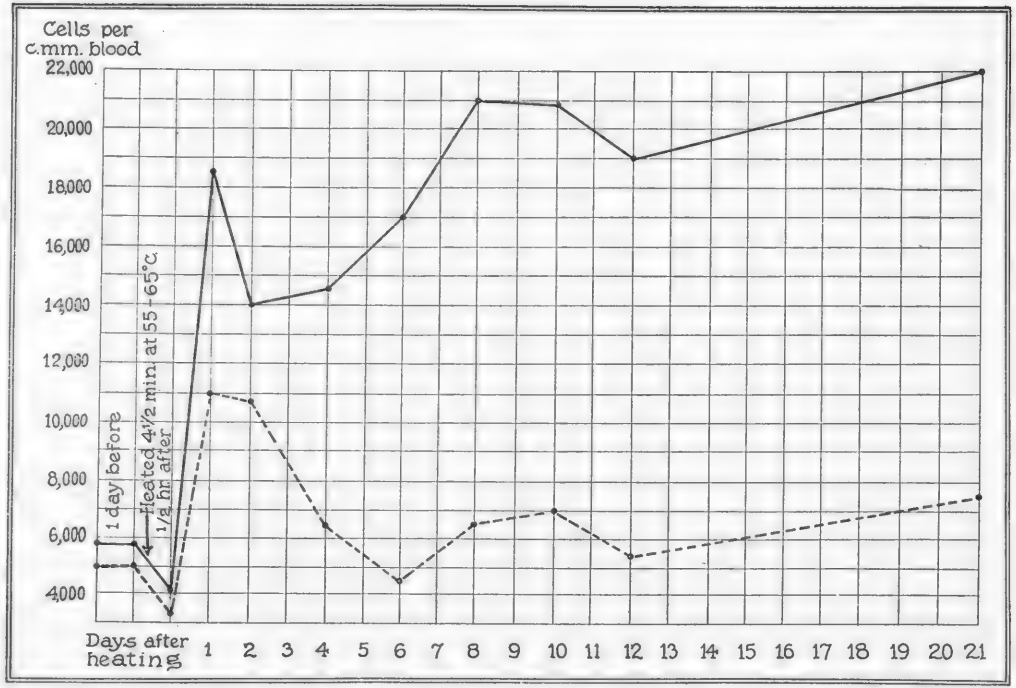
slight variation, showed the same characteristic drop in the blood count after heat was applied. After another 7 day interval the counts on Group I, 14 days after heating, showed an increase of 14,000 lymphocytes per c.mm. over the original count. Group II, 7 days after heating, showed an increase of 12,000 lymphocytes over the two previous counts. 14 days later Group I, 28 days



TEXT-FIG. 3. Composite curve of the white blood cell counts on two groups of mice, five in each group. One lot was heated a week after the first count, the others were heated 2 weeks after the first count with one intervening count. ————Lymphocytes. .....Polymorphonuclear leucocytes.

after heating, showed a count practically returned to its normal level, while Group II, 21 days after heating, was still several thousand cells above the normal count. This experiment is graphically illustrated in Text-fig. 3.

Experiment 3.—White blood counts were made on twenty healthy mice. 1 week later the entire twenty animals were heated for 5 minutes from 55–65°C.



TEXT-FIG. 4. Composite curve of the white blood cell counts on twenty healthy mice before and at intervals after heating. The first count represents the average for the whole lot. After heating they were counted in groups of five animals, the first group immediately after heating, the next 24 hours later, and then at intervals indicated on the chart. The last count represents the average of all twenty mice. ————Lymphocytes. .....Polymorphonuclear leucocytes.

and were then divided into four groups of five animals. Leucocyte counts were made on the first group immediately after heating, on the second 24 hours after heating, on the third 48 hours, and on the fourth 4 days after heating. Counts were made again on each group 6, 8, 10, and 12 days respectively after heating and again on all the animals 3 weeks after heating. The average of the counts for each day is shown graphically in Text-fig. 4, giving a continuous curve for the whole group.

Experiments 4, 5, and 6.—Three other experiments were carried out on mice in the same manner as those described above, with no variation in the result. These will not be given in detail.

In six experiments on mice it was found that after exposure of the animals to dry heat at 55–65°C. for 5 minutes there was a distinct fall in the total white blood cell count, both the polymorphonuclear leucocytes and the lymphocytes. The polymorphonuclear leucocytes recovered rather slowly, while the lymphocytes increased so rapidly that by the 2nd week after heating the count often reached a point 200 to 300 per cent above the normal figure.

The rectal temperature of mice after exposure to heat as described in the above experiments varies considerably, as shown by Table I.

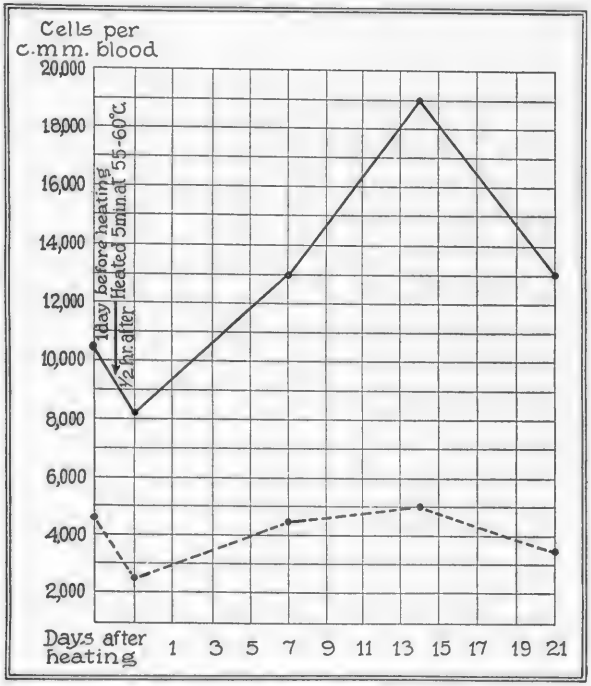
TABLE I.

Experiment No.	Before heat.	Directly after heat.	½ hr. after heat.	1 hr. after heat.
	°C.	°C.	°C.	°C.
1	36.2	39.1	38.3	37.7
2	36.7	39.0	37.2	37.0
3	36.6	39.7	37.1	35.1
4	36.0	41.6	35.7	35.2
5	37.2	40.8	38.3	37.7

Morphology of Circulating Lymphocytes after Heating.

No attempt has been made in this study to differentiate the large and small lymphocyte, as it is practically impossible to establish a satisfactory dividing line between the two types. In general it may be said that the larger type predominates in the earlier stages of the stimulation, while later the smaller ones increase. The cells of both types are for the most part normal, healthy looking lymphocytes, and do not differ in appearance from those observed in the

normal animal. At a period of from 6 to 10 days after heating it was noted that a proportion of the lymphocytes were in the process of what appeared to be amitotic division. Every stage (Figs. 1 and 2) of this division could be observed. In some of the more extreme cases there were found from three to five cells in one microscopic field (Fig. 3).

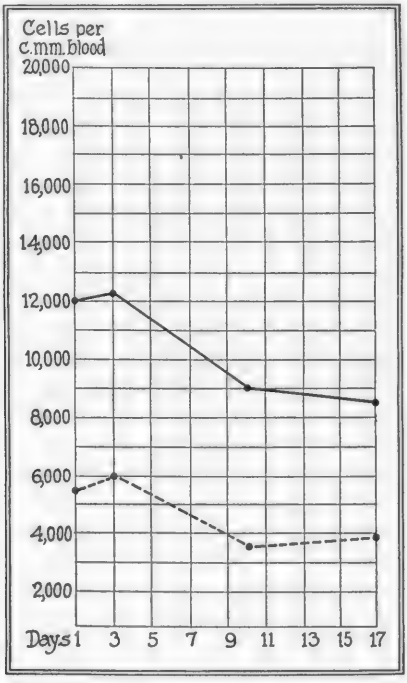


TEXT-FIG. 5. Composite curve of the white blood cell counts on five rats before and after heating. ————Lymphocytes. .....Polymorphonuclear leucocytes.

Effect of Heat on the Blood Picture in Rats and Guinea Pigs.

Experiment 7.—Blood counts were made on ten healthy rats of about the same age and size. The following day five of these animals were heated for 5 minutes at 55–60°C., counts being made immediately afterward both on these and on five control animals. The heated rats showed a decrease in both types

of cells similar to that observed in mice. The controls showed no marked change in their count. 7 days later counts showed the lymphocytes in the heated animals to be markedly increased, with the polymorphonuclear cells practically returned to their normal level. Control animals at this period showed a slight falling off in the count. 14 days after heating there was an average rise of over

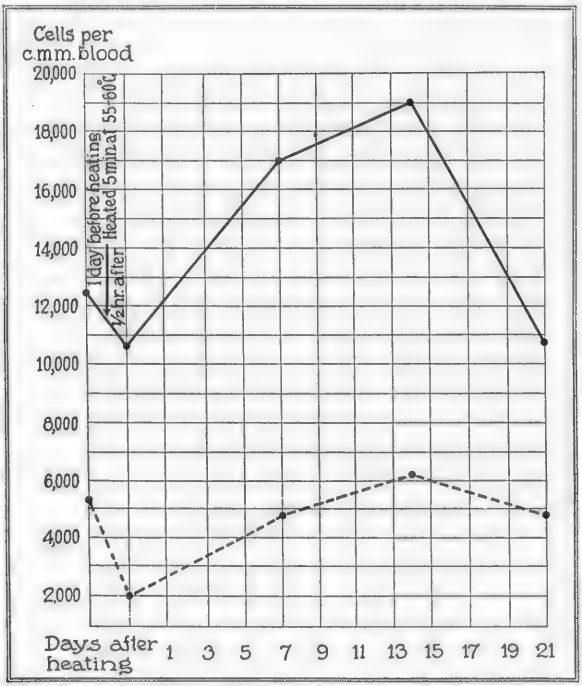


TEXT-FIG. 6. Composite curve of the white blood cell counts on five normal rats of the same lot as those in Text-fig. 5, kept under the same conditions, with counts made at the same time. ————Lymphocytes. .....Polymorphonuclear leucocytes.

8,000 cells per c.mm. of blood, while the polymorphonuclear count remained about the same. 21 days after heating, the lymphocytes showed a tendency to decrease, but still remained well above the normal level. Text-fig. 5 is the composite curve of the heated animals and Text-fig. 6 that of the controls. Text-fig. 7 shows the curve of a typical rat after heating.

Several other experiments of a similar character on rats gave identical results, the changes being similar in every way to those observed in mice.

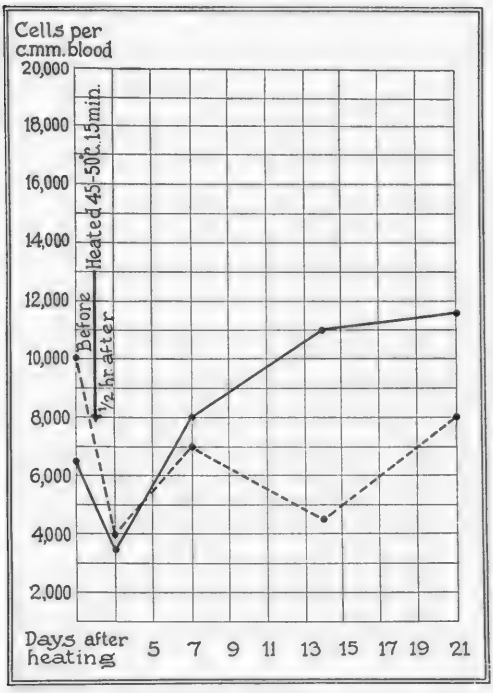
Experiment 8.—A total and differential leucocyte count was made on a fairly large guinea pig. The following day the animal was heated for 15 minutes at



TEXT-FIG. 7. Curve of the white blood cell counts of an individual rat after heating. ————Lymphocytes. .....Polymorphonuclear leucocytes.

45–50°C. and a count made shortly afterward. The lymphocytes showed only a slight decrease, while the polymorphonuclear leucocytes were reduced by 6,000 cells per c.mm. After this, counts were made at 7 day intervals for 3 weeks. They showed a steady increase in the lymphocytes, while the polymorphonuclears slowly regained their normal level and remained at that point (Text-fig. 8).

Several other experiments on guinea pigs gave identical results.



TEXT-FIG. 8. The white blood cell counts of the guinea pig after exposure to heat. ————Lymphocytes. .....Polymorphonuclear leucocytes.

DISCUSSION.

Some years ago Wickline[13] made a study of the blood cells in American troops incidental to the complete physical examination made at intervals during residence in the Philippines. He showed that there was a marked increase in the relative and absolute number of mononuclear blood units, the increase being at the expense of the polymorphonuclear leucocytes. There was no marked change in the total white cell count. Chamberlain,[14] later, incidental to the study of the Arneth count in the tropics, confirmed and extended

[13] Wickline, W. A., *Mil. Surg.*, 1908, xxiii, 282.
[14] Chamberlain, W. P., *Mil. Surg.*, 1909, xxv, 48.

this interesting observation. There is not sufficient data, however, in the literature to determine either seasonal variation, the effect of altitude, or the various climatic conditions, to enable us to discuss our present results in relation to these observations in man. Neither are we prepared to discuss the underlying mechanism which brings about these remarkable changes in the blood picture from a single exposure to dry heat, nor will we attempt to offer an explanation for this at the present time. For purposes of further investigation it offers a method by which we can produce a marked and durable increase in the circulating lymphocytes, thus affording a further opportunity for the study of the function of these cells.

The second point of interest is the large number of lymphocytes found in the circulation in the process of what appears to be an amitotic division. The majority of biologists consider that amitotic division is a degenerative process, and they are inclined to cast doubt on the possibility of the development into normal functioning cells. In either case we know that after heating there is a large increase in what appear to be normal circulating lymphocytes of both the large and small type. At present it is impossible to say positively where they have arisen. It seems probable that there is a sufficient stimulation of the lymphoid centers to account for the increase.[15]

SUMMARY.

Animals subjected to dry heat for a short period show a sharp fall in the total white blood count, both the polymorphonuclear leucocytes and the lymphocytes taking part in the fall. Following this there is a slow recovery on the part of the polymorphonuclear leucocytes, which generally require several weeks to regain their normal number. The lymphocytes rise rapidly after the initial fall and continue to rise for 2 or 3 weeks. This increase often amounts to a gain of over 200 to 300 per cent above the normal count for the animal. The observations were made on mice, rats, and guinea pigs.

The circulating lymphocytes during the more active stage of stimulation after heating show numerous examples of amitotic division.

[15] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Microphotograph from a blood film of a mouse 6 days after an exposure to heat, showing various stages of amitosis in the lymphocytes.

PLATE 2.

FIG. 2. Drawing of various stages of amitosis seen in the lymphocytes of the blood of a mouse 8 days after exposure to heat.

PLATE 3.

FIG. 3. A single microscopic field from a blood film of a mouse 8 days after exposure to heat, showing four irregular lymphocytes.

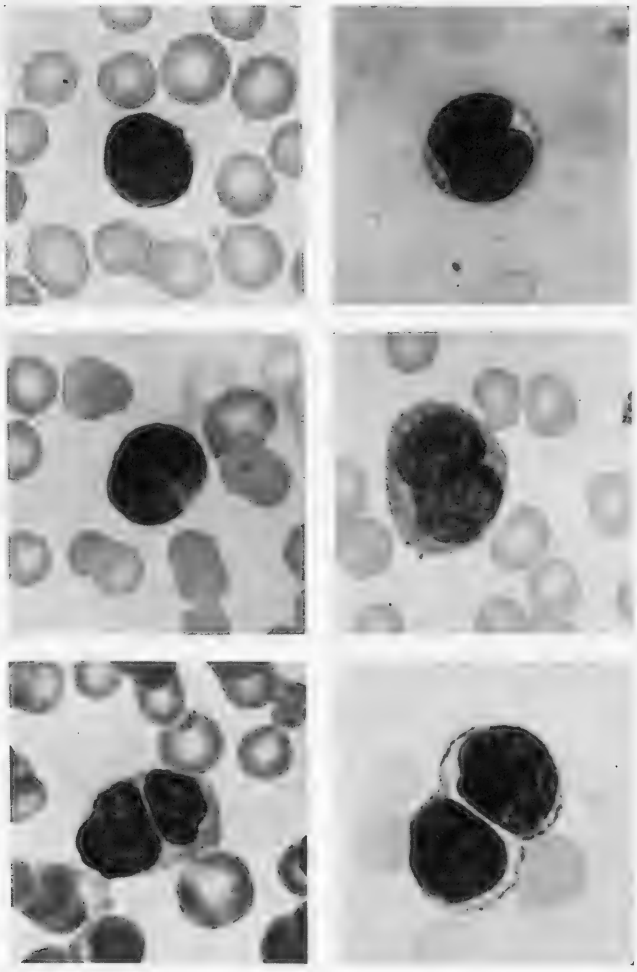


FIG. 1.

(Murphy and Sturm: Effect of dry heat on blood count. III.)

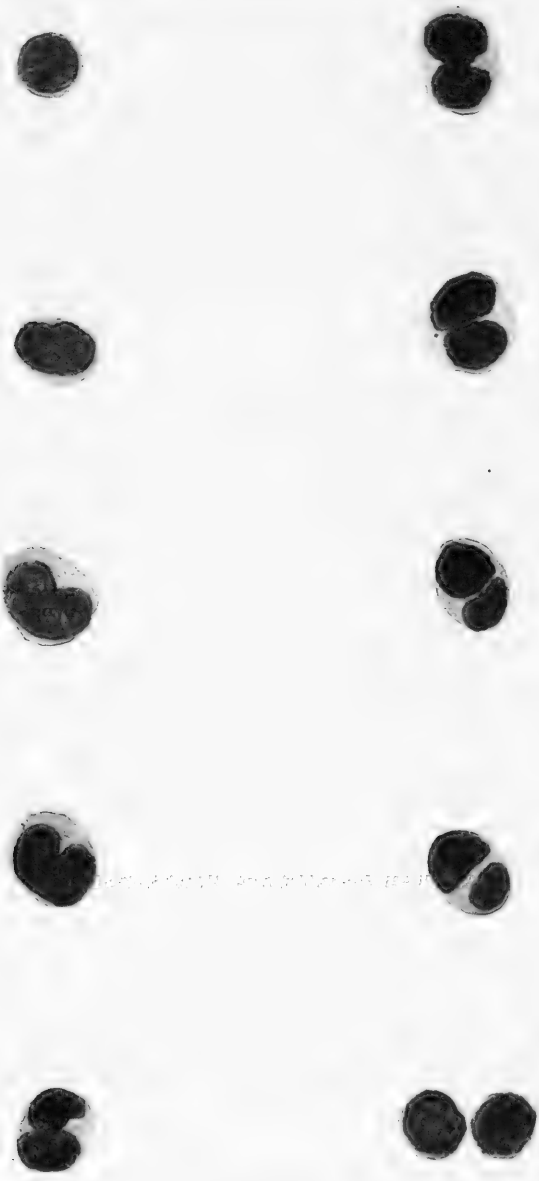


FIG. 2.

(Murphy and Sturm: Effect of dry heat on blood count. III)

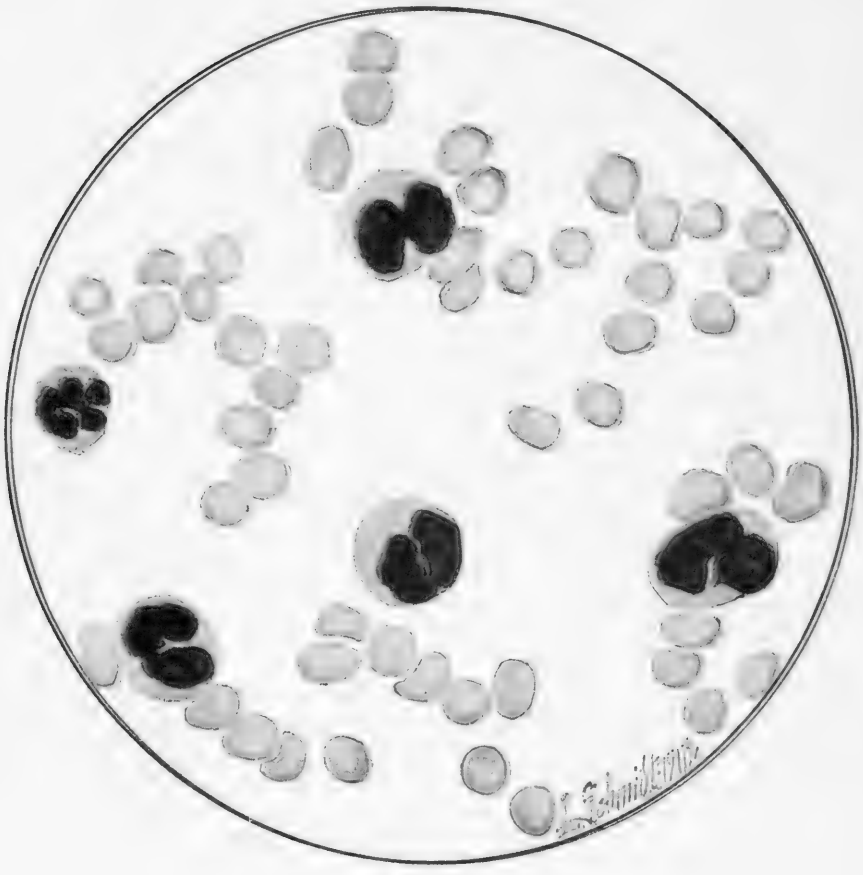


FIG. 3.

(Murphy and Sturm: Effect of dry heat on blood count. III.)

THE SOURCE OF THE LYMPHOCYTOSIS INDUCED BY MEANS OF HEAT.

By WARO NAKAHARA, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 4 to 7.

(Received for publication, October 17, 1918.)

A recent article from this laboratory reported experiments showing the effect of dry heat on the blood picture in mice.[1] After a 5 minute exposure of the animals to heat ranging from 55–65°C. there was an immediate fall in the circulating white cells. This was followed by a sharp rise in the lymphocytes which increased to a point about 200 per cent above their initial number. The increase extended over from 2 to 3 weeks and later gradually subsided to the normal level. The polymorphonuclear leucocytes participated in the initial fall but recovered very slowly and rarely went above the normal number. The lymphocytes resulting from this stimulation appeared to be normal, healthy cells of both the large and small type. It was noted that during the most active proliferative stage a number of lymphocytes were in the process of typical amitotic division. It seemed doubtful, however, in the light of the almost generally accepted opinion of biologists that this process ever gives rise to normally functioning cells. As the heat stimulation produces normal looking cells, it seemed desirable to study the other possible sources of origin.

Source and Type of Material.

The material for this study was collected by Murphy and Sturm[1] from a number of mice carried as a parallel to an experiment in which blood studies were made. These mice were of the same stock and about the same age. They were subjected to an exposure of dry heat for 5 minutes at a temperature ranging from 55–65° C. Several

[1] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.

of these mice were killed immediately after the heat exposure and the others in groups at intervals of 2, 4, 6, 8, 12, and 14 days. The spleen, lymph glands, and other organs were fixed in Flemming's, Bouin's, or Zenker's fluid. Sections were cut from 5 to 12 microns thick, and most of them stained with Heidenhain's iron-hematoxylin, eosin-methylene blue, and a few with Ehrlich's triacid stain. Of this material, the spleen and lymph glands have been studied and the results will be briefly described.

OBSERVATIONS.

Spleen.—The spleen becomes decidedly changed immediately after the treatment with heat. An examination reveals that there are a large number of cells degenerated or tending to degenerate (Fig. 1). Pyknosis is present not only in the cells of the nodules, but also in the pulp. The cell mass of the germinal center, however, seems always to remain unaffected (Fig. 2). There is no evidence of proliferative activity in the germinal center in the nodule, and only rarely can a mitotic figure be seen. There is also an apparent decrease in the number of the megalokaryocytes.

Sections taken 48 hours after the treatment present an appearance similar to that seen in the normal animals, the differences being an excessive number of necrotic cells, a relative scarcity of megalokaryocytes, and a great number of mitotic figures in the germinal center in the nodules. The active proliferation of cells in the germinal center (Fig. 3) is of significance in view of the fact that in the normal spleen of the adult the cells in the germinal center are not usually actively proliferating, while in infancy, when the lymphoid elements are actively formed, mitosis is of usual occurrence. These facts suggest that the occurrence of the mitotic figures in the germinal center, following the widespread degeneration, is primarily for the purpose of restoring the normal condition in the organ. The phenomenon may thus be regarded as one of overregeneration.

Sections taken 4 days after the treatment show that the spleen is well on the way to recovery (Fig. 4). Here necrotic cells are much less abundant, although they persist, especially in the pulp and in the periphery of the nodule. As in the normal spleen, the association

of mitotic figures with the germinal center is inconstant. The megalokaryocytes, which had been more or less decreased, are now present in fairly large numbers.

A condition similar to that last described is shown in all the sections taken later, after the treatment. That the megalokaryocytes are more, and necrotic cells less abundant in sections taken later may be looked upon as indicating complete recovery.

Macroscopically, the organ apparently undergoes marked enlargement at about the 6th to 8th day after the treatment. I have observed on the 8th day a spleen approximately four times the normal size. At the 14th day the size of the organ is seen to be normal. The size of the spleen varies greatly, even among apparently normal mice, but the fact of its enlargement after the treatment seems, in a general sense, to be indisputable.

Mesenteric Lymph Gland.—The cortex of the mesenteric lymph gland becomes full of necrotic cells immediately after the treatment (Fig. 5). The central cell mass of the nodules (germinal center) is apparently normal, but as a rule, no mitotic figures are seen in it. Numerous necrotic cells are observed in lymph cords, and no mitotic figures are seen. The pulp spaces are apparently normal.

After 48 hours the nodules are almost free of necrotic cells, although these cells are present in other parts of the cortex. Cells in the germinal center are actively proliferating, as evidenced by large numbers of mitotic figures in that region (Fig. 6). In lymph cords numerous necrotic cells and few mitotic figures are seen. The appearance of large multinucleated cells, probably phagocytic, is noted in the pulp spaces.

Conditions after the 4th up to the 14th day are apparently normal (Fig. 7). Mitosis in the germinal center is also of normal frequency, judging from the number encountered in sections.

Grossly, there is a general enlargement of the gland at about the 8th day after the treatment. The lymph glands taken at 14 days are approximately of normal size.

Inguinal Lymph Gland.—The changes observed here are exactly similar to those found in the mesenteric gland. Numbers of necrotic cells (Fig. 8) and suppression of the proliferative activity in the germinal centers are the fundamental characteristics immediately

after the treatment. After 48 hours necrotic cells become almost entirely eliminated from the cortex, and the germinal center assumes an appearance of great proliferative activity (Fig. 9). In the medulla there are still a number of necrotic cells persisting, but isolated cases of mitosis in lymph cords are not infrequent.

Histologically, the gland after the 4th day of the treatment is apparently normal. Here, also, as in the mesenteric gland, there is an apparent abnormal enlargement of the gland at about the 8th to 10th day after the treatment.

DISCUSSION.

One of the most striking findings in the observations described above is the complete parallel in the changes undergone by the splenic cells and the cells of the lymph glands. The first change observed in the spleen and in the two kinds of lymph glands, after the treatment with heat, is the widespread degeneration in their cell elements. Within 48 hours, or perhaps much earlier, the germinal centers become active, as demonstrated by the abundant mitotic figures in them, and they thus function in the restoration of the cellular elements of the organs. The germinal centers, however, later become inactive again.

In all the cases, both in the spleen and in the lymph glands, there is a marked increase of mitotic figures in the germinal center, following the general necrosis. The frequency of mitosis in the region apparently exceeds by far that in the normal animal, and it is natural to suppose that the enhanced cell proliferation accounts for the overproduction of lymphocytes. That the cell multiplication here is more than compensation for the degenerated cells is evident in light of the fact that dimensional enlargement of the organ, often well marked, follows the activity of the germinal center. The enlarged spleen and lymph glands are filled with normal cells, and it seems to be indisputable that excessive proliferation of the cells is responsible for their altered appearance. An attempt was made to ascertain whether there is definite increase in number and expansion in the area of germinal centers, after they are stimulated, but it has not been successful because of the unfavorable nature of the structure for such study.

It has been emphasized that a large number of cells in the spleen and in the lymph glands degenerate from the effect of heat immediately after its application. If, then, the cells in the spleen and lymph glands are a source of lymphocytes, there should be a fall in the number of these cells in the circulating blood following the application of heat. Murphy and Sturm have shown this to be the case. This corollary, taken together with other better known facts, tends to warrant the conclusion that the source of the induced lymphocytosis is at least partly the lymphoid cells in the spleen and lymph glands.

Results of recent experiments carried on in this laboratory point to the fact that a certain dose of x-rays can be used to produce a lymphocytosis similar to that brought about by heat.[2,3] The results of blood counts after suitable treatment of the animal with this agent prove that the lymphocytic change is parallel to that in the case of heat treatment, with the characteristic fall in lymphocyte count preceding the marked rise.

Attention may be called to the results of Heineke[4] and Warthin,[5] who have shown that the effect of x-rays on lymphoid tissues is, in the main, similar to that of heat, as described in the present paper. Their data, however, do not throw any light upon the question as to whether there is any proliferation of lymphoid cells above the normal in the process of regeneration, as they did not make observations during the critical period after the x-ray treatment, when an excessive multiplication of the cells may possibly take place. For its satisfactory solution, therefore, the problem must be reinvestigated from a standpoint different from that of the earlier workers.

The induced lymphocytosis is not due to the direct action of heat on lymphoid cells, but is due to proliferative activity on the part of the cells. In other words, heat is primarily a destructive agent of the lymphoid cells, but it causes secondary multiplication of the cells, thus bringing about pronounced lymphocytosis.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[3] Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

[4] Heineke, H., *Mitteil. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

[5] Warthin, A. S., *Physician and Surg.*, 1907, xxix, 1.

SUMMARY AND CONCLUSION.

A striking number of mitotic figures have been observed in the germinal center of the spleen and lymph glands during the regeneration of the cellular elements of these organs after the destructive effect of heat. This enhanced cell proliferation is interpreted as more than compensating for the degenerated cells, because of the subsequent enlargement of the organs. It has also been pointed out that the characteristic decrease in the number of lymphocytes immediately after the heat treatment is always accompanied by an extensive cell degeneration in spleen and lymph glands at the corresponding period.

On this basis it seems evident that the pronounced lymphocytosis induced by means of heat treatment of the animal is due, at least in part, to the enhanced proliferative activity of germinal centers in the spleen and lymph glands, reacting to the destructive effect of heat upon lymphoid cells.

EXPLANATION OF PLATES.

PLATE 4.

FIG. 1. A part of the spleen, immediately after the treatment, showing necrotic cells around the inactive germinal center. × 450.

FIG. 2. The same, showing normal cells of the germinal center (above), and necrotic cells around it (below). × 1,000.

PLATE 5.

FIG. 3. Germinal center of the spleen, 48 hours after the treatment. *M*, mitotic figures. × 1,000.

FIG. 4. Germinal center of the spleen, 4 days after the treatment, showing the condition to be apparently normal. × 1,000.

PLATE 6.

FIG. 5. Part of cortex of the mesenteric lymph gland, immediately after the treatment, showing marked necrosis. × 1,000.

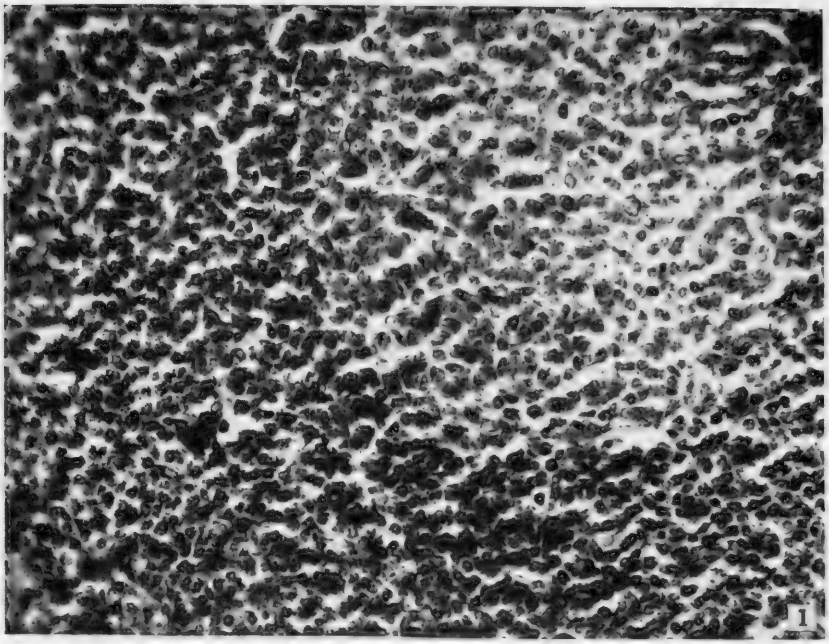
FIG. 6. Germinal center of the mesenteric lymph gland 48 hours after the treatment. *M*, mitotic figures. × 1,000.

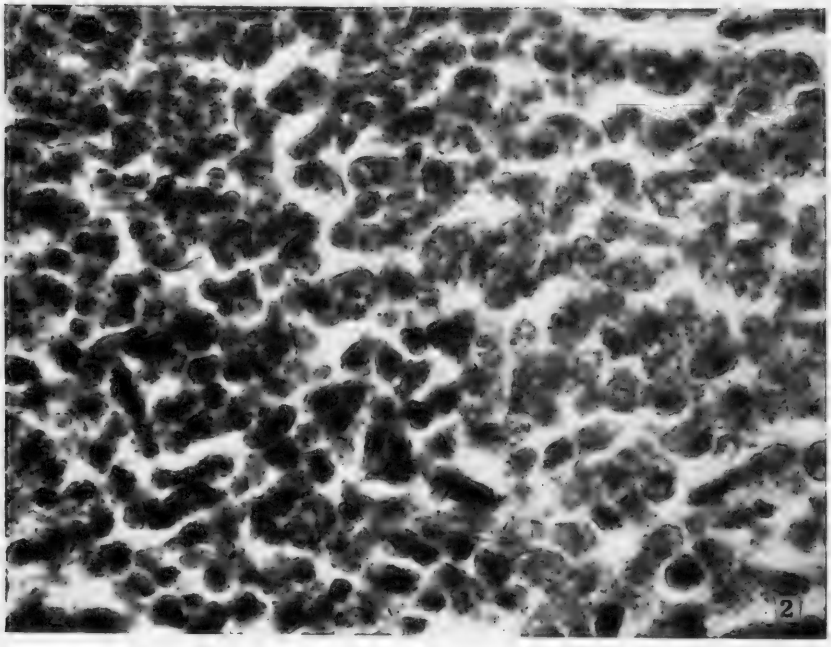
FIG. 7. The mesenteric lymph gland 12 days after the treatment, showing the germinal center (left) and cells around it. × 1,000.

PLATE 7.

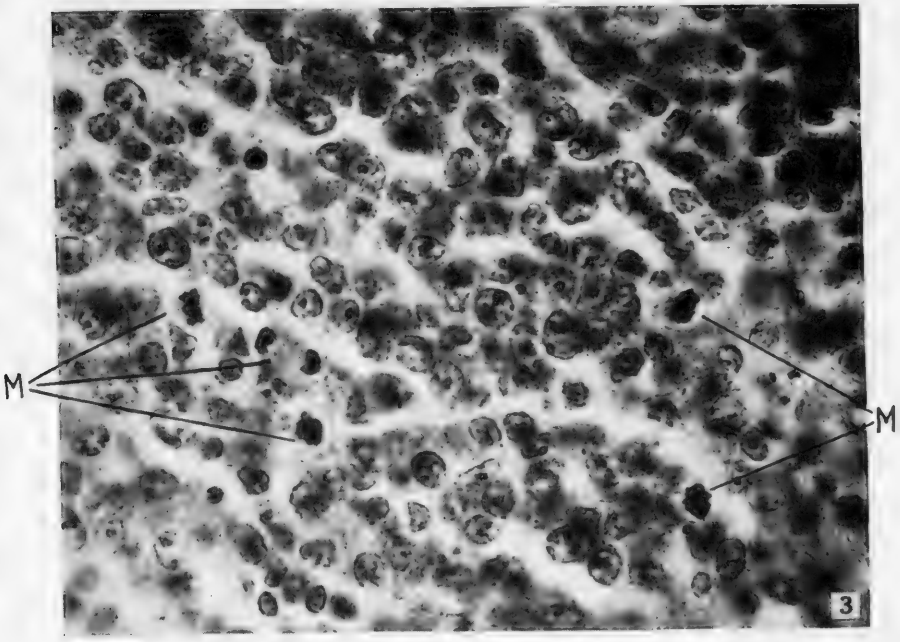
FIG. 8. Part of the medulla of the inguinal lymph gland, immediately after the treatment, showing necrotic cells. × 1,000.

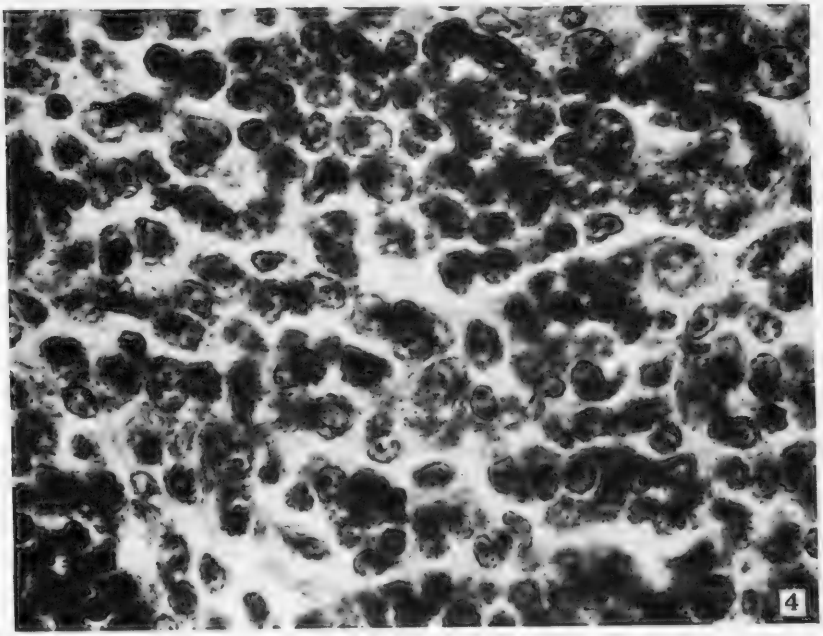
FIG. 9. Germinal center of the inguinal lymph gland 48 hours after the treatment. *M*, mitotic figures. × 1,000.



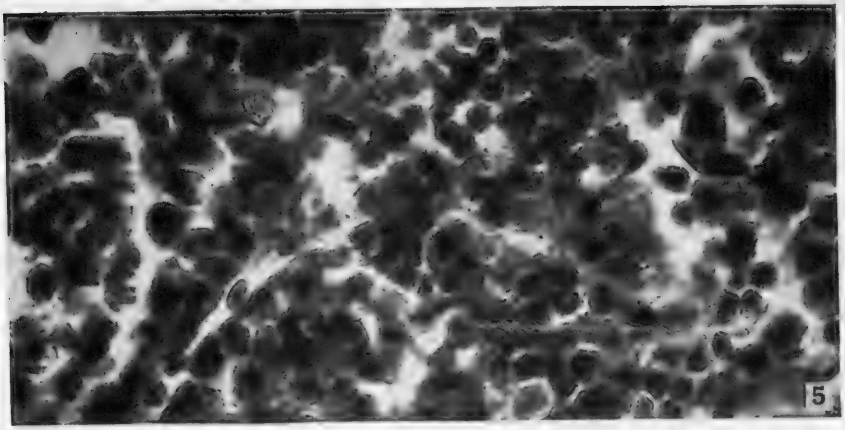


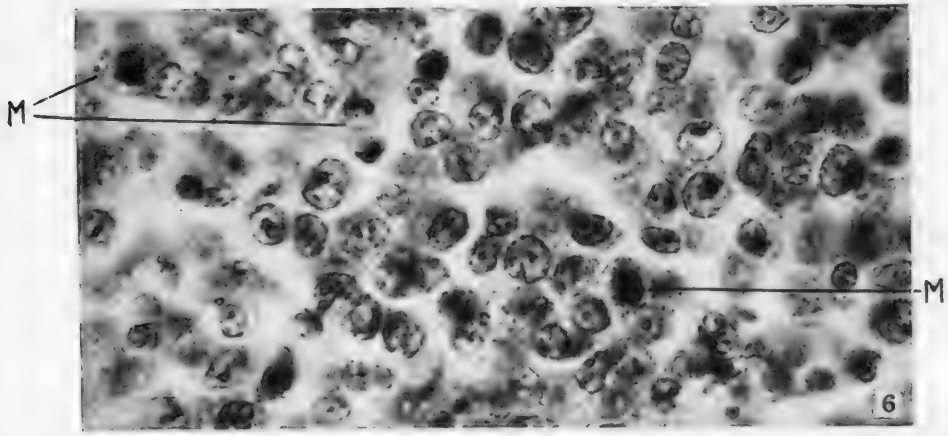
(Nakahara: Lymphocytosis induced by means of heat.)

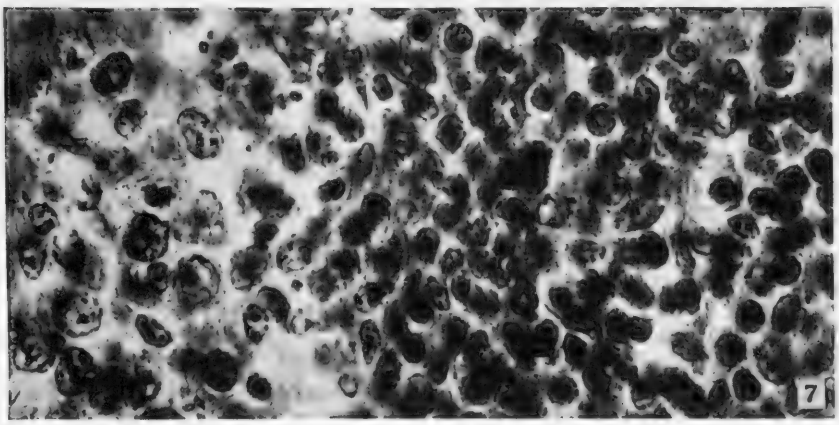




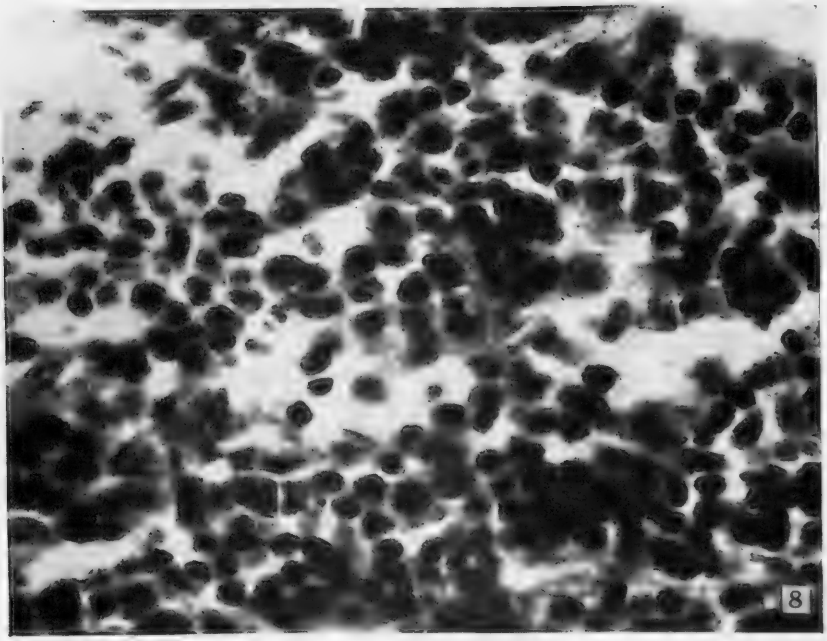
(Nakahara: Lymphocytosis induced by means of heat.)

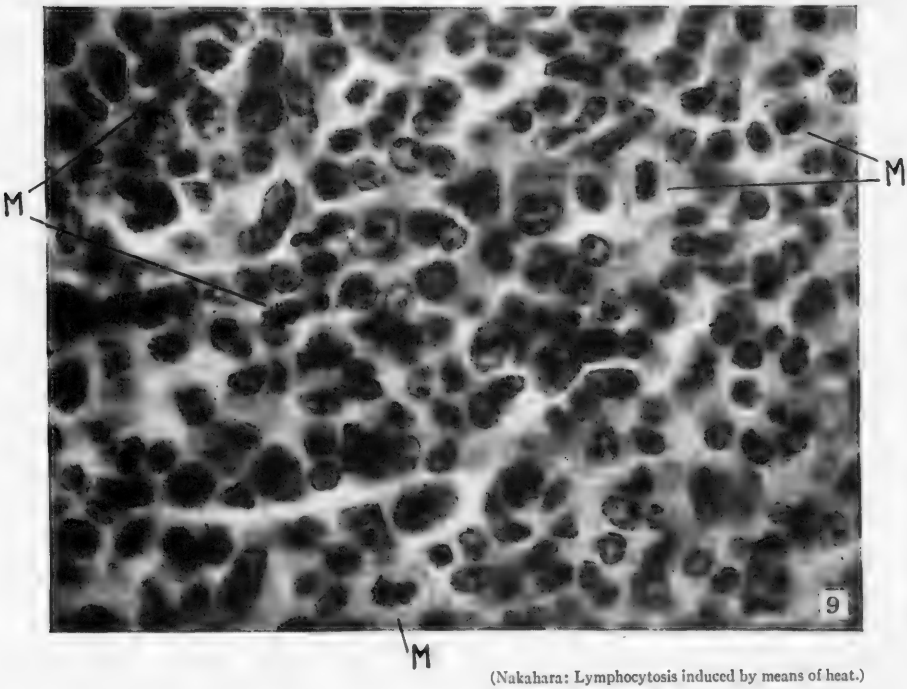






(Nakahara: Lymphocytosis induced by means of heat.





(Nakahara: Lymphocytosis induced by means of heat.)

THE LYMPHOCYTES IN NATURAL AND INDUCED RESISTANCE TO TRANSPLANTED CANCER.

IV. Effect of Dry Heat on Resistance to Transplanted Cancer in Mice.*

By JAMES B. MURPHY, M.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 18, 1918.)

The striking histological difference between a cancer graft in an immune animal and in a susceptible animal is the early appearance of large numbers of lymphocytes in the former and the relative absence of these cells in the latter. This fact has led some observers to venture the opinion that the lymphocytes may be a factor in the resistance to these growths. Da Fano[1] carried out extensive histological studies of this reaction about the cancer graft in immune animals and also extended his observations to changes in the cells of the subcutaneous tissue of the body. He demonstrated a marked increase in the lymphoid elements about the cancer graft and in the tissues, and likewise showed an increase in the closely related group of plasma cells. Later Murphy and Morton[2] showed that mice potentially immune to cancer developed a marked lymphocytosis after inoculation with a cancer graft. These investigators also showed that potentially immune animals can be rendered susceptible to cancer inoculation if the lymphoid tissue is depleted by means of x-rays. Murphy and Taylor[3] extended the latter observation and showed that immune animals of a tested resistance can be made again susceptible to inoculation after depletion of the lymphoid tissue.

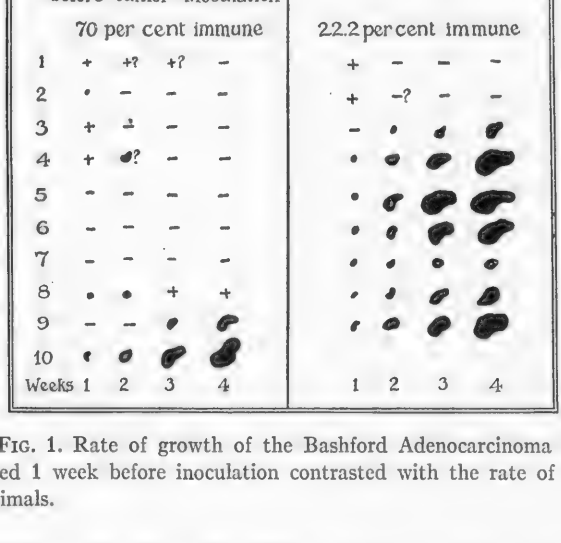
*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Da Fano, C., *Z. Immunitätsforsch., Orig.*, 1910, v, 1.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1.

It has been deemed advisable to test the effect of induced lymphocytosis in the animal on resistance to inoculated cancer. The amount of stimulation of the lymphocytes induced by splenectomy[4] and small doses of x-rays[5] did not prove sufficient to influence markedly the course of these highly resistant transplantable tumors. However, with the development of the more extensive and enduring stimulation induced by heat we have an opportunity of testing the effect of such a reaction on cancer resistance.



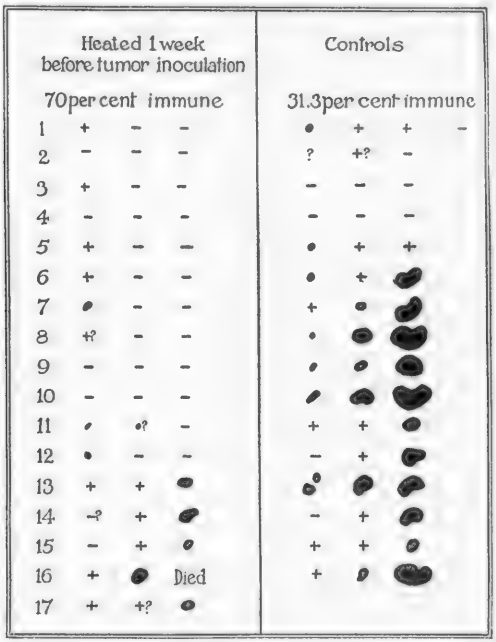
TEXT-FIG. 1. Rate of growth of the Bashford Adenocarcinoma No. 63 in mice heated 1 week before inoculation contrasted with the rate of growth in control animals.

Experiment 1.—Ten mice of about the same age and weight were heated for 5 minutes at 55–65°C. over an electric heat lamp. 1 week later the animals, together with ten healthy controls, were inoculated subcutaneously in the groin with a strain of a 3 week old transplantable cancer (Bashford Adenocarcinoma No. 63). The rate of growth of the tumors was charted at weekly intervals thereafter. At the end of 4 weeks the heated animals showed only three tumors, with an immunity of 70 per cent. Of the nine control animals surviving, seven developed tumors, and only two were immune. This per cent of immunity (22.2)

[4] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[5] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

is in striking contrast to the 70 per cent in the heated animals. The results of this experiment are shown in Text-fig. 1.

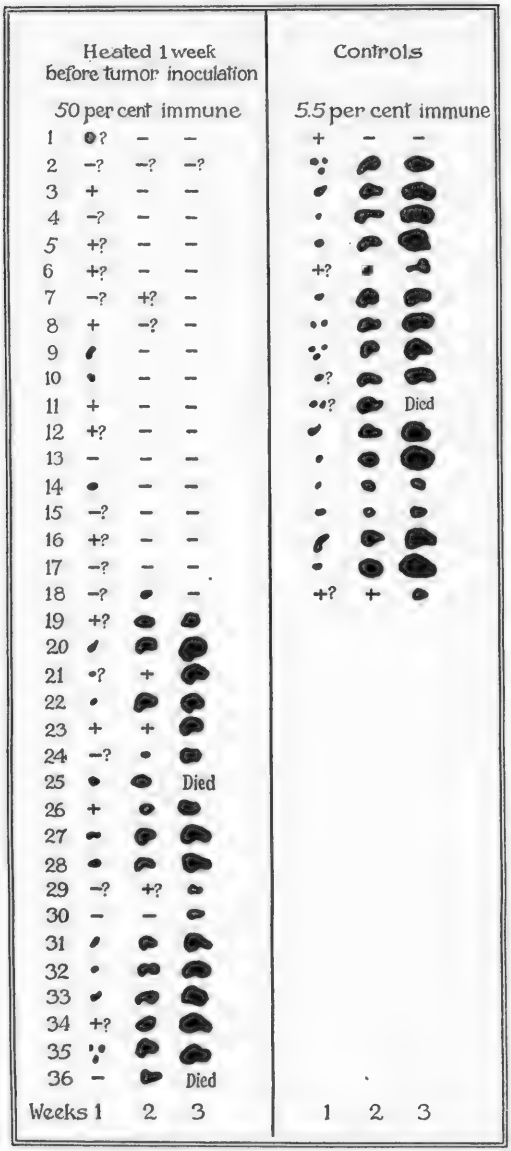
Experiment 2.—Seventeen mice were heated in the manner described in the previous paragraph. A week later these animals, with sixteen control mice, were inoculated with a strain of the Bashford Adenocarcinoma No. 63. Weekly measurements were made of the developing tumors. The heated animals showed five out of seventeen with tumors, or an immunity of 70 per cent. Eleven of the sixteen control animals developed tumors, the immunity being 31.3 per cent.



TEXT-FIG. 2. The same as Text-fig. 1.

The difference in immunity between the heated animals and the controls presents a striking contrast. The result is shown in Text-fig. 2.

Experiment 3.—Thirty-six healthy mice of about the same age and size and from the same stock were heated for 5 minutes over an electric heat lamp at a temperature ranging from 55–65°C. A week later these animals, with eighteen normal mice, were inoculated with a 2½ week old Bashford Adenocarcinoma No. 63. After 3 weeks eighteen of the thirty-six heated animals had developed tumors, showing an immunity of 50 per cent, while seventeen of the eighteen con-


Heated 1 week before tumor inoculation
50 per cent immune
Controls
5.5 per cent immune
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Died
Weeks 1 2 3
1 2 3

TEXT-FIG. 3. The same as Text-fig. 1.

trols had developed tumors, the immunity being only 5.5 per cent. This experiment is shown graphically in Text-fig. 3.

The three foregoing experiments, carried out on over 100 mice, show that animals whose lymphocytes have been stimulated by dry heat have a much higher resistance to transplanted cancer than control mice inoculated with the same tumor. The general health of animals subjected to this treatment did not seem in the least affected. There was no loss of weight, no roughening of the hair, or other indication of disturbance. This difference in resistance was manifest both when the tumor inoculated gave a relatively low per cent of takes, and when the tumor was highly virulent and gave a high per cent of takes.

DISCUSSION.

The absence of any acceptable demonstration of antibodies to explain cancer immunity suggests strongly that this type of resistance probably comes under the head of cellular immunity. The evidence connecting the lymphocyte with the resistance to transplantable cancer may be summed up briefly as follows: (*a*) the presence of lymphocytes and related cells about a cancer graft in immune animals, and the relative absence of these cells around such a graft in highly susceptible animals; (*b*) the general changes which take place in the cellular elements of the tissues of animals potentially immune to cancer (Da Fano); (*c*) the lymphocytic crisis in the circulating blood of potentially immune animals after inoculation with cancer; (*d*) destruction of potential cancer immunity by depletion of the lymphoid elements with x-rays; (*e*) destruction of established cancer immunity by the same means; (*f*) the marked increased resistance to cancer after artificial stimulation of the lymphocytes.

It would be difficult to adapt the foregoing facts so that they would fit either into the Ehrlich theory of cancer immunity or that of the Bashford school of stroma reaction. Neither of these explanations seems tenable in the light of the present results. That other factors than the lymphocytes are involved in the process of cancer immunity seems more than probable. It can at least be said with a degree of certainty that we have in the lymphoid elements an important link in the process of so called cancer immunity.

SUMMARY.

The marked and durable stimulation of the lymphoid elements induced by dry heat applied to the animal results in the establishment of a high degree of immunity to certain transplantable cancers in mice. This immunity is evident when the tumor used gives a low, as well as when it gives a high percentage of takes.

EFFECT OF STIMULATION OF THE LYMPHOCYTES ON THE RATE OF GROWTH OF SPONTANEOUS TUMORS IN MICE.*

By JAMES B. MURPHY, M.D., and ERNEST STURM.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 21, 1918.)

Murphy and Morton[1] have shown that mice with spontaneous tumors when exposed to small doses of x-rays sufficient to stimulate somewhat the lymphocytes exhibit an increased resistance to replants of their own tumors. In these experiments the tumors were removed by operation and with the tumor out the animal was exposed to a suitable small dose of x-rays. Immediately afterwards a graft of the original tumor was reinoculated into the groin of the mouse. Another series of mice with spontaneous tumors from the same strain was submitted to the same procedure, except that the x-ray treatment was omitted. 50 per cent of the x-rayed mice showed no growth of the returned graft or local recurrence of the cancer. The other 50 per cent showed a growth of the graft which appeared at a much later period than in the untreated animals. In the control group of animals, in which the x-ray treatment was omitted, over 96 per cent of the animals showed a growth of the returned graft. The latter figure corresponds to that obtained by other observers with similar experiments.

In the work to be reported here we have used dry heat as the method of stimulation instead of x-rays. A mouse exposed for 5 minutes to dry heat at a temperature ranging from 55–63°C. will show at first a slight fall in the circulating lymphocytes, followed by a marked increase, lasting from 2 to 4 weeks.[2] Accompanying the increase in

* This investigation was carried out by means of funds from the Rutherford Donation.

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[2] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix 1.

the circulating lymphocytes there is a marked activity on the part of the lymphoid organs evidenced by numerous mitotic figures in the germinal centers.[3]

Experiment.—The animals for this experiment were procured from the Lathrop stock.[4] There were 61 mice showing numerous gradations and types of mammary carcinomas. The tumors were removed as completely as possible by operation, and while the tumor was out the animal was heated for 5 minutes over an electric heat lamp at a temperature of 55–63°C. Immediately after heating, a graft of the original tumor was reinoculated subcutaneously into the left groin of the animal. In 36 of the 61 animals so treated a complete immunity to recurrence of the growth of the graft resulted. There were no metastases observed in any of these 36 animals. Only those mice were included in the series which lived for a period of over 4 weeks and remained in good condition. The majority lived for a much longer period than this. Among the 25 animals of this series which were not immune, the average time for the graft to become large enough to be recognized was 2 weeks and 5 days. The number of recurrences was seven. In some instances there was a recurrence which later retrogressed, but these have not been recorded as immune animals.

For controls we used our former series of animals which were taken from the same stock and were treated in the same way except that they were not heated. Of twenty-nine animals the returned graft grew in twenty-eight, and the average time required for the graft to become palpable was 1 week and 5 days. Local recurrences were present in fourteen out of the twenty-nine animals. Table I gives a comparison of the two series.

TABLE I.

Series No.*	Immune.	Susceptible.	Local recurrences.	Grafts alone.	Questionable grafts.	Metastasis alone.	Time for appearance of graft.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
I	59.4	40.6	11.3	14.7	8.1	6.5	2 wks. and 5 days.
II	3.4	96.6	48.3	48.3			1 wk. " 5 "

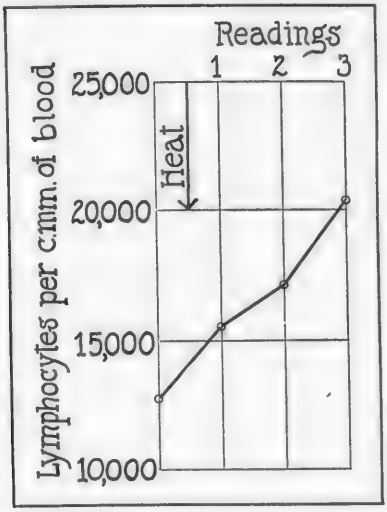
* Series I. 61 mice with spontaneous cancers heated after the removal of the tumor with later a return of a graft.

Series II. 29 control mice with cancer removed at operation and later a return of a graft.

[3] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

[4] The Lathrop stock of mice was recently purchased for The Rockefeller Institute for Medical Research by funds from the Rutherford Donation.

Blood counts were made on about half of the animals of the treated series, one before operation, and the next 1 week after operation and treatment, and subsequent counts were made at weekly intervals (Text-fig. 1). The average number of lymphocytes per c.mm. of blood before operation was about 12,000. 1 week after operation and heating they had risen to approximately 16,000, and they continued to increase until by the fourth count there were 20,000 lymphocytes per c.mm. of blood. In twenty of thirty-eight animals counted the polymorphonuclear leucocytes were lower after treatment than before. In the other eighteen, except in two or three cases where extensive infection occurred, there



TEXT-FIG. 1. The average number of lymphocytes of mice with spontaneous tumor after having been subjected to an exposure of heat. The counts were made before and at weekly intervals after heating.

was only a slight gain. These figures correspond with those reported for normal animals after exposure to heat. Counts made on untreated animals subjected to the same surgical procedure showed no such change.

DISCUSSION.

Pathologists from an early date have noted that lymphocytes accumulate about the slowly growing cancers, while they are absent in the more rapidly growing malignant types. This point has received a certain amount of comment but slight attempt has been

made at explanation. Our present results suggest that this infiltration may be of more importance than it has been previously considered. The great obstacle to overcome in accepting the lymphocyte as a factor of resistance to cancer growth is the fact that the lymph glands are the common point of metastases. This fact needs further study and elucidation but is not to be looked upon as an insurmountable barrier. Such possible questions present themselves as: What is the condition of the glands to which metastases take place? Is the resistance offered overcome by the number of cancer cells lodging in the lymph spaces? Are the lymphocytes in the gland in an actively functioning stage or do they require an activating substance like the opsonins? It is impossible to discuss these points with our present knowledge. The experiments reported here, coupled with the previous ones with x-rays, suggest strongly that the lymphoid tissue does offer a resisting influence to cancer growth, but this conception cannot be accepted unreservedly until further light is thrown on the activities of the lymph glands.

SUMMARY.

Spontaneous cancers were removed from a series of mice by operation. The animals were then subjected to an exposure to dry heat at a temperature ranging from 55–63°C. for 5 minutes. Immediately afterwards a graft of the original tumor was returned. The mice so treated exhibited a marked increase in their resistance to the growth of the cancer graft, over 59 per cent remaining entirely free from a return of the cancer. In a control series in which no treatment was given 96 per cent of the animals showed a return of the cancer.

EXPERIMENTS ON THE RÔLE OF LYMPHOID TISSUE IN THE RESISTANCE TO EXPERIMENTAL TUBERCULOSIS IN MICE.

III. Effect of Heat on Resistance to Tuberculosis.

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(Received for publication, October 19, 1918.)

In a previous report from this laboratory a series of experiments was recorded which seemed to explain the results of Lewis and Margot,[1] who noted that splenectomized mice had a greater resistance to tuberculosis than normal mice. Murphy and Ellis[2] demonstrated the fact that splenectomized mice, if exposed to suitable doses of x-rays no longer had an increased resistance, but were hypersusceptible to the infection. The interpretation of these results suggested, was that the increased resistance in splenectomized mice was due to the increase in the circulating lymphocytes, which was demonstrated to reach its height about 21 days after removal of the spleen. When the increase in the lymphocytes was prevented by x-rays, the increased resistance to tuberculosis was nullified. It was likewise shown that intact animals could be rendered less resistant to tuberculosis than controls by the use of broken doses of x-rays. The latter observation was confirmed by Morton[3] for the human strain of the organism with guinea pigs. Later Taylor and Murphy[4] showed that mice with a marked increase in the lymphocytes resulting from cancer inoculation in cancer-immune animals had also a marked increase in their resistance to tuberculosis. In this instance also if the increase in lymphocytes was de-

[1] Lewis, P. A., and Margot, A. G., *J. Exp. Med.*, 1914, xix, 187.

[2] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.

[3] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.

[4] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.

stroyed by x-rays, the animals were rendered highly susceptible to infection.)

We regarded these results, coupled with the deductions from observations of the blood count in men with this disease, and the histology of the lesion, as strong direct evidence that the lymphocytes play an important part in the resistance of the animal to tuberculosis. With the development of a new method of stimulating the lymphocytes,[5] namely that of intense dry heat, we have another opportunity to test this conception.

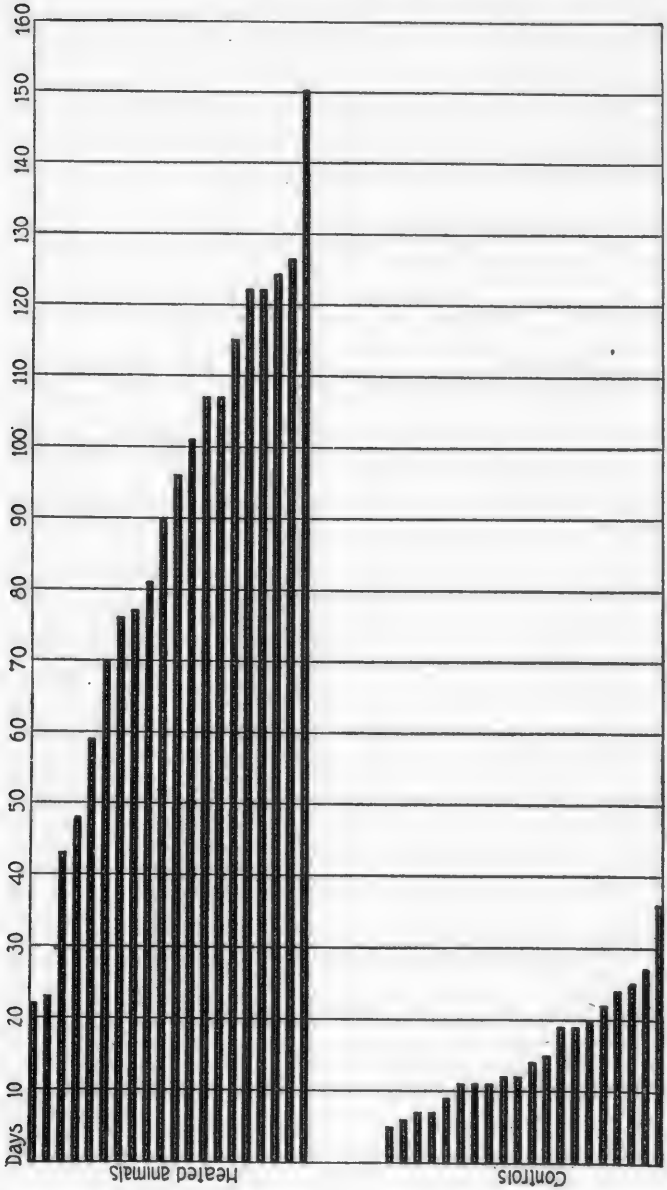
Method.

Mice subjected to dry heat ranging from 55–65°C. over a period of 5 minutes first show a fall in the total white blood count, both the polymorphonuclear cells and, to a lesser extent, the lymphocytes participating in the fall. The lymphocytes, however, show an immediate rebound, followed by a rapid increase which carries the count several hundred per cent above the normal. This rise continues for 14 to 21 days, after which there is a gradual return to the normal level. The polymorphonuclear leucocytes recover slowly and show no stimulation phase. This method gives us an excellent opportunity to test the effect of a marked increase in the lymphocytes on the course of tuberculous infections.

Experiment 1.—Forty mice of the same stock and of about the same age and size were selected for this experiment. Twenty were heated over an electric heat lamp for 5 minutes at a temperature starting at 55°C. and allowed to increase to 65°C. 1 week later these animals, together with the twenty controls, were each inoculated intraperitoneally with 2 mg. of a bovine strain of tubercle bacilli (4 week culture) suspended in 0.5 cc. of normal salt solution. The mice were then placed in individual jars in order to prevent the occurrence or spread of an epidemic. All the mice as they died were carefully autopsied, and films were taken from the peritoneal fluid, and from liver, kidney, lungs, and heart's blood. It was found that all the animals had widely disseminated tuberculosis, which could definitely be accepted as the cause of death.

The control animals died rapidly, the first one on the 6th day, and the last on the 36th day after inoculation. The average number of days of life for this group was approximately 16 days. None of the heated animals died until the

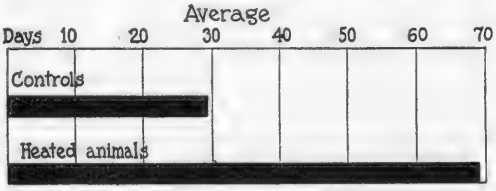
[5] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.



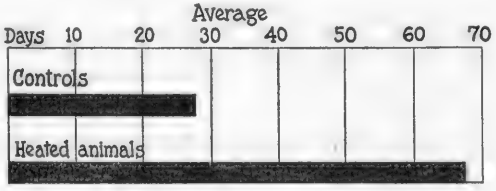
TEXT-FIG. 1. Each horizontal line represents the time of survival of a mouse after inoculation with bovine tubercle bacilli. The first group was subjected to an exposure of dry heat 1 week before inoculation. The second group was untreated.

22nd day after inoculation, and the longest period of life in this group was 150 days, the average length of life for the twenty mice being approximately 88 days. The rate at which the animals died is shown in Text-fig. 1.

Experiment 2.—Twelve healthy mice of about the same age and weight were heated in the same manner as in the previous experiment. A week later these, with twelve control mice from the same stock, were inoculated intraperitoneally with 2 mg. of a bovine strain of the tubercle bacillus suspended in 0.5 cc. of normal salt solution. The animals were segregated in individual jars as in the previous experiment. Autopsies were performed promptly after death and films taken from the principal organs, the peritoneal fluid, and the heart's blood.



TEXT-FIG. 2. The average duration of life of heated mice after inoculation with bovine tubercle bacilli compared with the duration of life of untreated animals. There were twelve mice in each group.



TEXT-FIG. 3. The average duration of life of heated mice after inoculation with bovine tubercle bacilli compared with the duration of life of untreated animals. There were twelve heated mice and fourteen controls.

All these mice showed extensive tuberculosis except one control which lived 89 days, and which was either a highly resistant animal or was not infected because of some accident in the inoculation. In this series the heated animals averaged 69 days of life after inoculation, while the controls averaged only 29 days. The details of this experiment are shown in Text-fig. 2.

Experiment 3.—Twenty-six mice of the same strain and about the same age were selected for the experiment. Twelve were subjected to direct heat ranging from 55–65°C. for 5 minutes. A week later each of the twenty-six animals was inoculated intraperitoneally with 2 mg. of a culture of the bovine tubercle bacillus suspended in 0.5 cc. of normal salt solution. They were segregated in separate

jars as in preceding experiments. Autopsies were performed as the animals died to verify the cause of death. In all, except one of the control animals, there was widely disseminated tuberculosis. In this animal, which lived 117 days, there was no evidence of the disease.

In this experiment the heated animals averaged 67.6 days of life after inoculation, while the controls lived only 27.8 days. The rate at which the mice died is shown in Text-fig. 3. In the last two experiments the same culture of the tubercle bacillus was used for inoculation.

The three experiments described show that animals subjected to one exposure of dry heat and inoculated a week later with a bovine strain of the tubercle bacillus virulent for mice had a greatly enhanced resistance to the organism as compared with that of the untreated animals. The difference in resistance here is much more striking than that seen in splenectomized animals or in the cancer-immune animals.

DISCUSSION.

The impression is gathered from the literature that individuals recovering from tuberculosis develop an increase in the number of circulating lymphocytes, although the point is rarely emphasized. In an analysis of a number of blood counts on rapidly advanced cases of tuberculosis in man a marked decrease in this type of cell is observed. The fact that between these two characteristic findings there is every variation in the type and degree of reaction, with often practically normal blood counts, has led to skepticism in regard to the significance of the blood changes in tuberculosis. In dealing with such a slow, chronic infection, the changes would not be expected in a marked degree except in the two extremes mentioned above. The blood changes, together with the fact that the tuberculous lesion is characterized by an accumulation of large numbers of lymphocytes, are suggestive of the part played by this cell in resistance to tuberculosis. To this evidence we add the results of our experiments; namely, the lowering of resistance to tuberculosis in animals depleted of their lymphocytes by means of x-rays, and the increased resistance in animals with the lymphocytes increased by three widely different methods, splenectomy, cancer immunity, and dry heat.

The chief points which may be brought against this conception is that tuberculosis frequently involves the lymphoid structures, and

they seem, if anything, more susceptible to the infection than many other tissues. The explanation of this fact is not clear, but we do not consider it an overwhelming argument in the face of the mass of evidence which indicates that the lymphocyte is an important factor in the resistance to the tubercle bacillus.

SUMMARY.

Mice with high lymphocyte counts and increased activity of the lymphoid tissue induced by one exposure to intense dry heat exhibit a marked increase in the resistance to large doses of bovine tubercle bacilli as compared with that shown by control animals given a similar inoculation. This resistance, judged by the time of survival after inoculation, is increased from two- to threefold. The average length of life after inoculation for three groups of heated mice was 88, 69, and 67 days respectively, while the control groups averaged 16, 29, and 28 days respectively.

EFFECT OF EXPOSURE TO THE SUN ON THE CIRCULATING LYMPHOCYTES IN MAN.

By HERBERT D. TAYLOR, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, June 12, 1918.)

Experiments reported previously[1-4] indicate that in animals a blood lymphocytosis attends increased resistance to cancer and tuberculosis. The work has shown that the number of circulating lymphocytes can be varied by suitable dosage of the Roentgen rays. Massive x-ray exposures decrease, while small ones increase, both proportionately and actually, the number of lymphocytes in the circulating blood. It seemed possible that the beneficial results of heliotherapy in surgical tuberculosis noted by Rollier and others[5] might be due, in part at least, to a similar effect of the actinic rays on the lymphoid organs, leading to an increase in the number of circulating lymphocytes. The fact that heliotherapy is applied chiefly at high altitudes in the Alps suggested also that the actinic rather than the heat rays were the therapeutic agents. The ultraviolet rays contained in light of solar origin lie in that portion of the invisible spectrum included between 4,000 and 2,950 Ångström units.[6] Light waves of lengths included between 2,100 and 2,800 and, to a lesser extent, those up to 4,000 Ångström units are bactericidal.[7] However, as shown by Newcomer as well as by previous observers, this action is lost in the presence of organic matter in minute traces. Inasmuch as the surgical lesions treated successfully in this manner

[1] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.

[2] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[3] Morton, J. J., *J. Exp. Med.*, 1916, xxiv, 419.

[4] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.

[5] For a review of the subject see: Roatta, G. B., L'Elioterapia nella pratica medica e nell 'educazione, Milan, 1914.

[6] Bovie, W. T., *Bot. Gaz.*, 1915, lix, 149.

[7] Newcomer, H. S., *J. Exp. Med.*, 1917, xxvi, 841.

are deep seated and the ultra-violet rays have but feeble penetrating power, it does not seem probable that a direct destructive effect on their bacterial causes is responsible for the clinical improvement reported. Fresh air, good food, and rest, in the absence of sunlight do not produce a corresponding effect.

In order to determine whether exposure to the sun has any effect on the circulating lymphocytes of healthy individuals, the following study was made.

A series of blood counts was made, in the spring of 1916, on several individuals who expected to be exposed to the sun during the ensuing summer months at Woods Hole, Massachusetts. In the fall a second series of counts was made on the same subjects, and this report is based on observations made on the bloods of 38 persons who showed more or less evidence of chronic solar dermatitis (tanning) at the time the final blood count was made. Most of the individuals studied were tanned over a large portion of their bodies, and no instances are included in which there was tanning merely of the face and hands. The subjects included both sexes and ranged in age from 20 to 65 years. It is admitted that tanning of the skin is caused by actinic and not by heat rays.

Method.

The blood to be counted, obtained from a needle prick in the tip of the finger, was diluted 1:20 with 3 per cent acetic acid solution, the mixture shaken in a diluting pipette for 5 minutes, a drop placed on a Türck hemocytometer, and an interval allowed for the corpuscles to settle. The white corpuscles in 8 of the 9 ruled millimeter squares of the counting chamber were counted and the number per c.mm. was estimated in the usual way. Films made at the same time on coverslips were used for differential leucocyte counts. After staining with Wright's stain, 300 cells were counted and the percentage of each type was determined. The total number of lymphocytes per c.mm. of blood was estimated by multiplying the total number of white corpuscles per c.mm. by the percentage as determined by differential count.

The results of the study, for convenience arranged in Tables I to VIII, are analyzed in the discussion which follows.

Discussion of Tables.—A study of the tables brings out the fact that the blood of persons in whom chronic solar dermatitis is present shows not infrequently considerable percentage and actual increase of the lymphocytes. Thus in 25, or 65.8 per cent of the 38 persons

TABLE I.

Individuals Showing an Increase in Lymphocytes after Exposure to Sun.

Individual No.	Lymphocytes.		Increase.
	Before exposure to sun.	After exposure to sun.	
	per cent	*per cent*	*per cent*
1	28.1	43.8	55.5
2	26.3	31.7	20.8
3	33.0	39.0	18.1
4	19.6	25.3	29.0
5	30.0	32.0	8.6
6	26.0	36.0	38.5
7	28.0	34.0	21.4
8	22.6	32.6	44.2
9	35.0	42.6	21.7
10	30.3	37.0	22.1
11	38.6	47.3	22.5
12	20.0	22.0	10.0
13	22.6	29.6	30.9
14	26.0	35.0	36.9
15	19.6	42.2	115.3
16	33.6	47.2	40.5
17	27.1	33.0	21.8
18	22.0	25.6	16.4
19	28.0	33.6	20.0
20	23.6	25.9	9.7
21	30.0	42.8	42.7
22	28.0	31.8	13.6
23	34.6	39.0	12.7
24	26.0	42.4	63.1
25	27.0	39.6	46.6
Average.....	27.4	35.6	27.2

studied, there was a definite increase in the number of circulating lymphocytes after development of solar pigmentation which averaged 27.2 per cent. This increase, given in the column headed "Increase" in Table I, represents the percentile rise of lymphocytes above the

TABLE II.

Individuals Showing a Decrease in Lymphocytes after Exposure to Sun.

Individual No.	Lymphocytes.		Decrease.
	Before exposure to sun.	After exposure to sun.	
	per cent	*per cent*	*per cent*
26	34.6	32.2	6.9
27	42.3	33.0	21.3
28	35.5	28.0	21.1
29	41.3	29.1	29.5
30	42.0	36.3	13.6
31	42.3	37.6	11.1
32	43.8	42.7	2.5
33	34.6	32.6	5.8
Average.....	39.6	33.9	15.2

TABLE III.

Individuals Showing No Change in Lymphocyte Percentage after Exposure to Sun.

Individual No.	Lymphocytes.		Increase.	Decrease.
	Before exposure to sun.	After exposure to sun.		
	per cent	*per cent*	*per cent*	*per cent*
34	29.3	30.0	2.4	
35	33.3	33.0		0.9
36	18.0	17.6		2.2
37	27.5	26.9		2.2
38	27.0	27.8	2.9	
Average.....	27.0	27.0	5.3	5.3

TABLE IV.

Summary of Bloods Studied.

Individuals with.	No.	Per cent.
Lymphocytes increased after exposure to sun.....	25	65.8
" decreased " " " "	8	21.0
" showing no change after exposure to sun.....	5	13.2
Total.....	38	100.0

original percentage of these cells. In eight, or 21 per cent, there was an average decrease in the percentage of lymphocytes amounting to 15.2 per cent (Table II). In five, or 13 per cent, there was no substantial change in the lymphocyte percentage (Table III).

If one disregards for the moment the individuals, 5 in number, whose blood showed no definite percentage change, it follows that of the remaining 33, 25, or 73.8 per cent, exhibit an increase, and 8, or 24.2 per cent, a decrease of the circulating lymphocytes. The changes noted in the number of total lymphocytes before and after tanning tended to parallel the percentage change in the same blood. Thus (Table V) twenty-five bloods which gave a definite percentage lymphocyte increase gave also an average absolute increase from 2,574 to 3,338 cells per c.mm. Moreover, the eight bloods which

TABLE V.

Average Total Lymphocyte Counts.

Individuals (Tables I, II, and III.)	Average before exposure to sun.	Average after exposure to sun.	Increase.	Decrease.
			per cent	*per cent*
25 with percentage increase	2,574	3,338	29.6	
8 " " decrease	3,807	3,187		16.3
5 " no change in percentage	2,951	3,060	3.5	

gave a decrease in the percentage of lymphocytes followed the same trend, as the average number of these cells before exposure was 3,807, and after 3,187 per c.mm. It should be noted here that the bloods showing no appreciable change in percentage of lymphocytes gave an average total number per c.mm. which varied only slightly as the result of the exposure, the average before exposure being 2,951 and after exposure 3,060.

The average percentage change in the total lymphocytes per c.mm., after exposure, for each of the three series compares closely with similar determinations of the percentile increase or decrease as given in Tables I, II, and III. The twenty-five persons who showed an average percentile increase of 27.2 showed a total lymphocyte increase of 29.6 per cent. The percentile decrease in the eight individuals included in Table II was 15.2, and the average total lymphocyte

TABLE VI.

Complete Count on Individuals Showing an Increase in Lymphocytes after Sunburn.

Individual No.	White blood corpuscles.	Small lymphocytes.	Large lymphocytes.	Polymorphonuclear neutrophils.	Polymorphonuclear eosinophils.	Polymorphonuclear basophils.	Transitionals and large mononuclears	Lymphocytes.	Absolute lymphocytes.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
1	8,648*†	24.2	3.8	66.1	3.2	0.1	2.4	28.1	2,377
	6,850	40.8	3.0	48.0	3.5	0.8	3.8	43.8	2,978
2	7,720	20.6	5.7	65.5	1.7	1.3	5.2	26.3	2,048
	8,550	27.7	3.8	60.4	3.1	0.6	3.9	31.7	2,725
3	9,710	28.5	4.5	59.8	2.0	0.8	4.3	33.0	3,105
	6,920	34.0	5.0	58.0	0.6	0.3	2.0	39.0	2,739
4	13,450	15.6	4.0	76.3	0.0	0.3	3.6	19.6	2,636
	10,900	23.3	2.0	67.3	3.6	0.3	3.3	25.3	2,758
5	7,390	26.5	3.5	65.0	1.0	3.8	0.3	30.0	2,213
	8,240	27.6	5.0	65.0	0.3	0.0	1.6	32.6	2,687
6	15,900	22.3	3.6	69.3	2.3	0.0	2.3	26.0	4,134
	7,159	32.8	3.5	53.7	1.7	0.3	8.0	36.0	2,675
7	9,650	25.3	2.6	62.0	3.0	0.0	7.0	28.0	2,702
	7,100	29.3	4.7	57.5	4.2	0.2	4.2	34.0	2,419
8	10,750	20.0	2.6	75.3	0.0	0.3	1.6	22.6	2,430
	9,750	31.0	1.6	63.0	1.6	0.3	2.3	32.6	3,180
9	7,250	31.3	3.6	56.0	4.0	0.6	4.3	35.0	2,538
	5,050	40.0	2.6	46.0	4.6	0.0	6.6	42.6	2,151
10	14,750	26.6	3.6	57.3	8.3	1.3	2.6	30.3	4,469
	8,875	34.3	2.7	53.8	4.8	0.8	3.5	37.0	3,330
11	8,250	37.0	1.6	55.6	2.6	0.0	3.0	38.6	3,185
	11,750	43.3	4.0	46.6	1.3	0.0	4.6	47.3	5,558
12	9,700	16.6	3.3	77.3	0.3	1.3	1.0	20.0	1,940
	11,850	21.3	0.6	68.6	5.0	0.3	4.0	22.0	2,607

*Average counts are given in Tables VI to VIII because in many instances from two to six counts were made before, and a similar number after exposure to the sun.

†The upper row of figures for each individual represents counts before, and the lower row counts after exposure to the sun.

TABLE VI—*Concluded.*

Individual No.	White blood corpuscles.	Small lymphocytes.	Large lymphocytes.	Polymorphonuclear neutrophils.	Polymorphonuclear eosinophils.	Polymorphonuclear basophils.	Transitionals and large mononuclears.	Lymphocytes.	Absolute lymphocytes.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
13	10,667	20.6	2.0	67.0	9.3	0.3	0.6	22.6	2,410
	14,500	25.3	4.3	59.3	5.3	0.3	5.3	29.6	4,292
14	7,800	23.0	3.0	59.0	1.6	1.0	2.3	26.0	2,028
	9,500	33.3	2.3	61.0	2.3	0.0	1.0	35.6	3,382
15	7,050	16.3	3.3	74.6	0.0	0.3	5.3	19.6	1,382
	6,200	39.6	2.6	52.0	0.0	0.6	5.0	42.2	2,616
16	6,000	29.0	4.6	58.6	3.0	1.0	3.6	33.6	2,016
	5,800	42.6	4.6	46.0	1.0	1.6	4.0	47.2	2,738
17	7,925	24.6	2.5	68.3	0.8	0.2	3.5	27.1	2,189
	9,600	30.3	2.6	60.3	2.0	0.3	4.3	33.0	3,168
18	6,700	17.5	4.5	70.0	4.0	0.0	4.0	22.0	1,474
	7,350	20.6	5.0	64.6	2.6	0.0	7.0	25.6	1,882
19	9,300	25.6	2.3	59.3	10.0	0.6	2.0	28.0	2,604
	11,100	32.6	1.0	59.6	3.3	0.3	3.0	33.6	3,696
20	6,850	21.0	2.6	72.0	0.6	0.3	3.3	23.6	1,617
	7,825	22.8	3.1	68.3	2.0	0.2	3.5	25.9	1,983
21	10,850	26.3	3.6	62.6	3.3	0.6	3.3	30.0	3,255
	9,550	40.3	2.5	47.3	6.0	0.5	3.3	42.8	3,886
22	9,100	25.6	2.3	65.6	2.3	0.0	4.0	28.0	2,548
	9,900	30.6	1.1	62.8	2.3	0.2	2.8	31.8	3,084
23	8,200	32.3	2.3	60.0	1.0	0.0	4.3	34.6	2,837
	8,900	35.6	3.3	53.3	2.0	1.0	4.6	39.0	3,471
24	12,778	25.0	1.0	69.6	1.6	0.0	2.6	26.0	3,322
	9,575	40.8	1.6	51.5	4.2	1.3	0.5	42.4	4,059
25	10,667	25.3	1.6	70.6	0.0	0.6	1.6	27.0	2,880
	12,550	38.3	1.3	56.3	1.0	1.0	2.0	39.6	4,970
Average....	9,482	23.9	3.1	65.7	2.6	0.6	3.1	27.4	2,574
	9,054	32.7	2.9	57.2	2.8	0.4	3.8	35.7	3,338

decrease was 16.3 per cent. Table III gives 0.0 as the average percentile change in the five persons included there, while the average total lymphocyte increase in the same group was 3.5 per cent.

TABLE VII.

Complete Counts on Individuals Showing a Decrease in Lymphocytes after Sunburn.

Individual No.	White blood corpuscles.	Small lymphocytes.	Large lymphocytes.	Polymorphonuclear neutrophilis.	Polymorphonuclear eosinophilis.	Polymorphonuclear basophilis.	Transitionals and large mononuclears.	Lymphocytes.	Absolute lymphocytes.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
26	8,683	29.9	4.8	58.9	0.6	0.7	5.0	34.6	2,973
	8,933	28.8	3.4	62.0	1.1	0.5	4.1	32.2	2,768
27	6,714	36.8	5.5	46.5	2.3	1.8	7.0	42.3	2,874
	7,400	30.3	2.6	62.6	0.0	1.0	3.3	32.0	2,442
28	6,249	30.5	5.0	58.6	1.3	0.4	4.2	35.5	2,199
	8,658	23.8	4.3	64.6	2.2	0.6	4.5	28.0	2,353
29	7,900	37.0	4.3	49.6	1.6	0.3	7.0	41.3	3,263
	10,250	26.3	3.2	59.5	3.0	0.6	7.2	29.1	2,972
30	12,750	37.0	5.0	55.0	0.6	0.3	2.0	42.0	5,355
	13,000	34.0	2.3	59.3	1.0	0.3	3.0	36.3	4,719
31	11,100	39.6	2.6	53.3	0.6	0.0	1.6	42.3	4,695
	9,050	36.0	1.6	56.6	0.0	0.3	5.3	37.6	3,403
32	6,850	39.5	4.3	47.8	1.8	1.2	5.3	43.8	3,000
	6,783	40.5	2.2	51.2	2.9	0.4	2.6	42.7	2,917
33	8,950	31.6	3.0	57.6	3.3	0.0	4.3	34.6	3,097
	8,950	28.3	4.3	63.0	2.3	0.0	2.0	32.6	2,918
Average....	8,650	35.2	4.3	53.4	1.5	0.6	4.6	39.6	3,807
	9,128	31.0	3.0	59.9	1.7	0.5	4.0	33.8	3,187

As may be seen from Tables VI, VII, and VIII there was no constant change in the total numbers of white blood corpuscles per c.mm. of blood as determined before and after exposure. A study of these tables also shows that the polymorphonuclear neutrophilic leucocytes tended to vary in inverse proportion with the lympho-

cytes, these cells decreasing when the lymphocytes increased and *vice versa*. The polymorphonuclear eosinophilic and basophilic (mast) cells and the group composed of the transitional and large mononuclear granular cells (bone marrow oxidase cells, Evans[8]) remained very constant.

TABLE VIII.

Complete Counts on Individuals Showing No Change in Lymphocytes after Sunburn.

Individual No.	White blood corpuscles.	Small lymphocytes.	Large lymphocytes.	Polymorphonuclear neutrophils.	Polymorphonuclear eosinophils.	Polymorphonuclear basophils.	Transitionals and large mononuclears.	Lymphocytes.	Absolute lymphocytes.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
34	15,600	26.3	3.0	63.6	1.0	1.0	5.0	29.3	4,571
	17,800	25.3	4.6	61.6	1.6	0.6	6.0	30.0	5,340
35	7,900	28.3	5.0	61.6	0.6	0.3	4.0	33.3	2,631
	7,850	31.0	2.0	61.3	1.3	0.6	3.6	33.0	2,591
36	11,350	14.3	3.6	77.0	2.0	1.0	2.0	18.0	2,043
	13,950	15.3	2.3	74.3	0.6	0.6	6.6	17.6	2,455
37	11,975	24.9	2.5	68.3	0.5	0.8	2.8	27.5	3,378
	9,750	24.7	2.1	67.4	2.4	0.5	2.6	26.9	2,600
38	7,900	24.3	2.6	61.6	6.0	0.0	5.3	27.0	2,133
	7,825	23.3	4.5	62.2	5.0	0.5	4.5	27.8	2,213
Average....	10,945	23.6	3.4	66.4	2.0	0.6	3.8	27.0	2,951
	11,435	23.9	3.1	65.4	2.2	0.6	4.7	27.1	3,060

Rollier and others[9] have emphasized the fact that tanning is necessary to obtain beneficial results in tuberculosis with heliotherapy. In this connection it is interesting to note that of the thirteen individuals who showed no increase in their circulating lymphocytes, six had the pasty type of skin which does not tan. Three others were so dark normally that it was impossible to determine whether or not there had been additional pigmentation. Five

[8] Evans, F. A., *Arch. Int. Med.*, 1916, xviii, 692.

[9] Rollier, A., etc., quoted in Roatta,[5] p. 19.

of the eight persons whose blood showed a loss in lymphocyte percentage had circulating cells of this type in excess of 40 per cent before exposure to the sun. These observations are interesting as offering possible explanations of the apparent exceptions to the general tendency to develop a definite lymphocytosis after chronic solar dermatitis. Thus the twenty-five persons with a definite increase in circulating lymphocytes were all tanned to a greater or less extent. The increase in lymphocytes, moreover, tended to be greatest in the individuals in whom the pigmentation was most increased, yet this was not invariably the case; so that the increase in lymphocytes and degree of tanning did not always go hand in hand so far as this series of tests is concerned.

In regard to complexion, no particular difference between the blood or skin response to solar irritation was noted with the exception of the three individuals in whom it was impossible to determine whether or not additional pigmentation was present. So far as observed age and sex had no effect on the blood response to light.

DISCUSSION.

From the results recorded here it seems that the rays contained in the solar spectrum, as it reaches the earth in the temperate zone in midsummer, bring about changes in the white corpuscles of the blood in a manner similar to that recognized as characteristic of the x-rays. The manner of action is equally obscure with regard to both agents.

Of interest in connection with this study are blood counts made by Chamberlain and Vedder[10] on Americans living in the Philippines. Although analyzed by them to ascertain the effect of the tropical climate on the Arneth blood picture, their counts show a higher percentage of lymphocytes than normal individuals in the temperate zone.[11] In the 72 cases reported there was an average of 38.6 per cent. The tropical service of the soldiers composing the series averaged 28.7

[10] Chamberlain, W. P., and Vedder, E. B., *Philippine. J. Sc.*, 1911, vi, 408.

[11] In a series of more than 100 observations on the bloods of 46 healthy individuals from 20 to 65 years of age, made in connection with this study, it was found that the lymphocytes averaged 29.3 per cent.

months. Wickline,[12] in counts made upon the blood of 104 soldiers 3, 12, and 19 months after arrival in the Philippines, showed that there was a definite and progressive increase in the mononuclear cells. These cells averaged 31.4 per cent at the first, 34.4 per cent at the second, and 39.5 per cent at the third determination. Although there is no distinction made between the mononuclear elements, tending to separate the lymphocytes from the cells belonging to the granular series, some idea of the direction in which the cells of lymphatic origin varied may be obtained by following the percentage of small mononuclear cells, which are undoubtedly the small lymphocytes. The average percentage of these cells at the first count was 21.8, at the second count 26.6, and at the third count 33.3. Disregarding the large lymphocytes, which should be combined with the small cells of the same series to give an accurate idea of their number at each determination, it can be seen that there was a definite increase of these cells in the blood after 19 months in the tropical climate. The soldiers were undoubtedly exposed to the sun for long periods of time. It also seems possible that the invisible spectrum is somewhat widened in the tropics[13] and there is consequently a wider range of ultra-violet rays than in the temperate zone. The intensity of the visible rays of the spectrum is also increased as we approach the equator. It is probable that the invisible rays increase likewise in proportion to those that are visible. Although the effect on the blood seems to be related to the ultra-violet rays of sunlight, it is impossible to rule out the effect of infra-red, or heat waves in these observations.

SUMMARY.

Chronic solar dermatitis was accompanied, in 25 of the 38 individuals studied, by an appreciable increase, percentage and absolute, in the number of circulating lymphocytes.

In eight there was a definite decrease and in five no appreciable change after prolonged exposure to the rays of the summer sun.

Of the thirteen subjects with no increase in blood lymphocytes, six

[12] Wickline, W. A., *Mil. Surg.*, 1908, xxiii, 282.

[13] Bovie, W. T., *Am. J. Trop. Dis. and Prev. Med.*, 1914–15, ii, 512.

failed to tan, three were so dark originally that to determine an increase was impossible, and five had an extremely high lymphocyte count at the first count.

Blood counts on white persons living in the Philippines indicate that the blood lymphocytes are likewise increased after a prolonged period of residence in the tropical zone.

Because of the parallelism between the tanning and the blood changes it seems probable that the lymphocytosis observed in the majority of instances, which is similar to the response of the blood of animals to small doses of the x-rays, is due to the effects of the ultra-violet rays contained in the solar spectrum.

STUDIES ON X-RAY EFFECTS.

I. Destructive Action on Blood Cells.*

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Pusey,[1] Senn,[2] Brown,[3] Bryant and Crane,[4] and others,[5,6] have reported on cases of leucemia treated with the x-rays and all seem to agree that there is a definite reduction of the circulating leucocytes, following treatment. Most of the cases reported were, however, of the splenomyelogenous type and the myelocytes were the cells most affected. Capps and Smith[7] treated lymphatic leucemia with the Roentgen rays and noted a marked reduction of the circulating lymphocytes, after treatment, in five of their six cases.

Heineke,[8] the first to make careful histological examinations of animals following x-ray exposures, demonstrated that the lymphatic tissues of the body were primarily affected. He found degeneration of the lymph follicles in the spleen and lymph glands and a diminution of circulating lymphocytes. He correlated his observations by suggesting that the diminution in circulating lymphocytes is directly referable to the selective destruction of the lymphogenic tissues by the x-rays.

Helber and Linser[9] published blood counts made on a rabbit before and after x-ray treatment. A marked percentile and absolute reduction in the circulating

*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Pusey, W. A., *J. Am. Med. Assn.*, 1902, xxxviii, 166.

[2] Senn, N., *N. Y. Med. J.*, 1903, lxxvii, 665.

[3] Brown, E. J., *J. Am. Med. Assn.*, 1904, xlii, 827.

[4] Bryant, B. L., and Crane, H. H., *Med. Rec.*, 1904, lxv, 574.

[5] For a review of the literature see Warthin, A. S., *Internat. Clin.*, 1906, iv, series 15, 243.

[6] For a further review of the literature see Pancoast, H. K., *Univ. Penn. Med. Bull.*, 1906–07, xix, 282.

[7] Capps, J. A., and Smith, J. F., *J. Am. Med. Assn.*, 1904, xliii, 981.

[8] Heineke, H., *Mitt. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

[9] Helber, E., and Linser, P., *Münch. med. Woch.*, 1905, lii, 689.
Linser, P., and Helber, E., *Deutsch. Arch. klin. Med.*, 1905, lxxxiii, 479.

lymphocytes occurred. These cells, before treatment, represented 32 per cent of the white blood corpuscles, a total of 2,080 lymphocytes per c.mm. of blood. After treatment they had decreased to 6 per cent, or but 120 cells per c. mm. In a white rat there was a reduction of lymphocytes from 60 per cent, or 8,400 cells before, to 30 per cent, or 1,020 cells after x-ray treatment. These results were confirmed by other experiments.

Warthin[5] confirmed Heineke's work and added further evidence indicating that the x-rays have a specific destructive action on lymphoid tissues. He found the Malpighian bodies of the spleen to be first affected and later the lymph glands and bone marrow. Histologically, he noted fragmentation of the tissue lymphocytes, the particles being ingested by phagocytes. Mitotic figures were absent or very infrequently encountered in the lymphogenic tissues following x-ray treatment. After a short interval the lymph follicles, when present, were either inconspicuous or invisible in gross, and but few lymphocytic elements were found microscopically. The stroma of the organs affected was much in evidence, owing to the destruction and consequent disappearance of the lymphogenic cells. Heineke and Warthin both employed x-rays in lethal doses.

It has been shown that resistance to cancer[10] and tuberculosis,[11] in animals, is attended by an increase in number of the circulating lymphocytes and that the resistance, as well as the lymphocytosis, may be destroyed by x-rays in proper dosage.[11, 12] Likewise the natural resistance of animals to heteroplastic tissue grafts, which seems to be associated definitely with a local accumulation of lymphocytes, may be destroyed by the x-rays.[13]

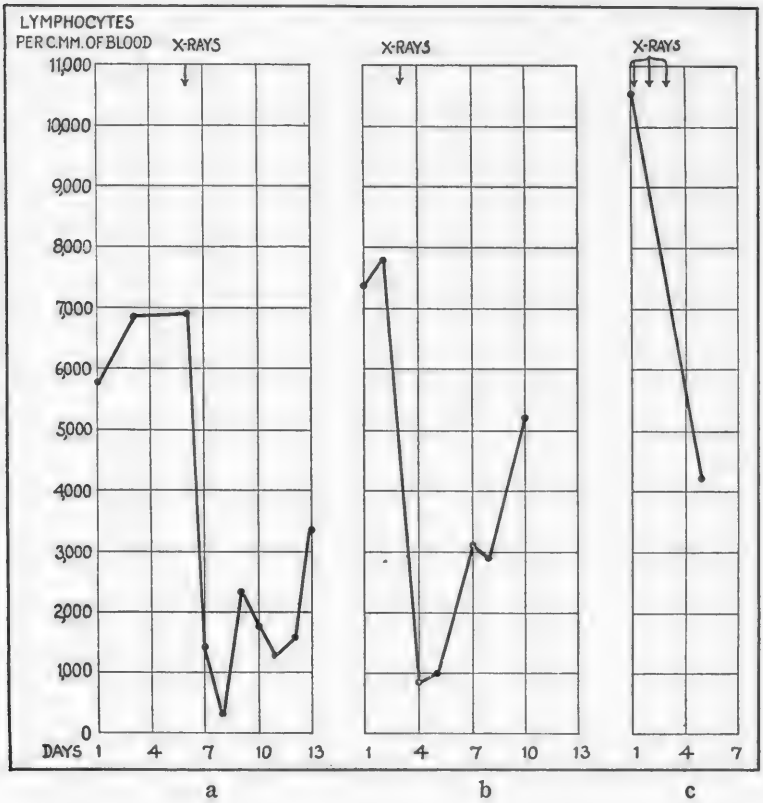
Inasmuch as (*a*) it is not possible to estimate the dosage employed in most of the older experiments concerning the effect of the x-rays on the blood, because gas tubes were used, (*b*) only a few blood counts have been published in these cases and their significance has not been adequately explained, (*c*) the x-rays are now being used with increasing frequency in therapeusis; it seemed important to obtain accurate information regarding the response of the blood to x-rays, and with this purpose the following results are recorded.

EXPERIMENTAL.

Experiment 1.—Eight areas, comprising both flanks of a Shetland pony, about 8 years old, were successively exposed in a single day to unfiltered x-rays generated by a Coolidge tube. The factors governing the dose at each exposure were:

[10] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.
[11] Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, xxv, 609.
[12] Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, xx, 397.
[13] Murphy, Jas. B., *J. Am. Med. Assn.*, 1914, lxii, 1459.

spark-gap 8 inches, milliamperes 5, distance from the target to the skin 12 inches, and time 10 minutes. By computing the dose[14] from the factors given, it was found that the animal received over each area 20, or over the eight areas 160 Holzknecht



TEXT-FIG. 1, *a*, *b*, and *c*. (*a*) The effect of x-ray treatment on the circulating lymphocytes of a pony. (*b*) The effect of x-ray treatment on the circulating lymphocytes of a cat. (*c*) The effect of x-ray treatment on the circulating lymphocytes of a guinea pig.

units. The total number of lymphocytes per c. mm. of blood at each observation, during the period of study, is charted in Text-fig. 1, *a*. The per cent

[14] Remer, J., and Witherbee, W. D., *Am. J. Roentgen.*, 1917, iv, 303.

and total number of lymphocytes and polymorphonuclear neutrophilic leucocytes per c. mm. of blood at each determination are tabulated in Table I.

Experiment 2.—A healthy adult monkey (*Macacus rhesus*) was given a series of unfiltered x-ray treatments, extending over a period of 8 days. Seven doses were given, the dorsal and ventral surfaces of the body being alternately exposed. The factors at each treatment were: spark-gap 3 inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time 4 minutes. This dose represents, at each exposure 6, or a total of 42 Holzknecht units. Text-fig. 2 graphically illustrates the effect of the x-rays on the circulating lymphocytes in this animal, each determination referring to the total number of lymphocytes per c. mm. of blood. In Table II the blood counts, made at different times in the course of the experiment, are summarized to show more fully the changes in the circulating cells following treatment.

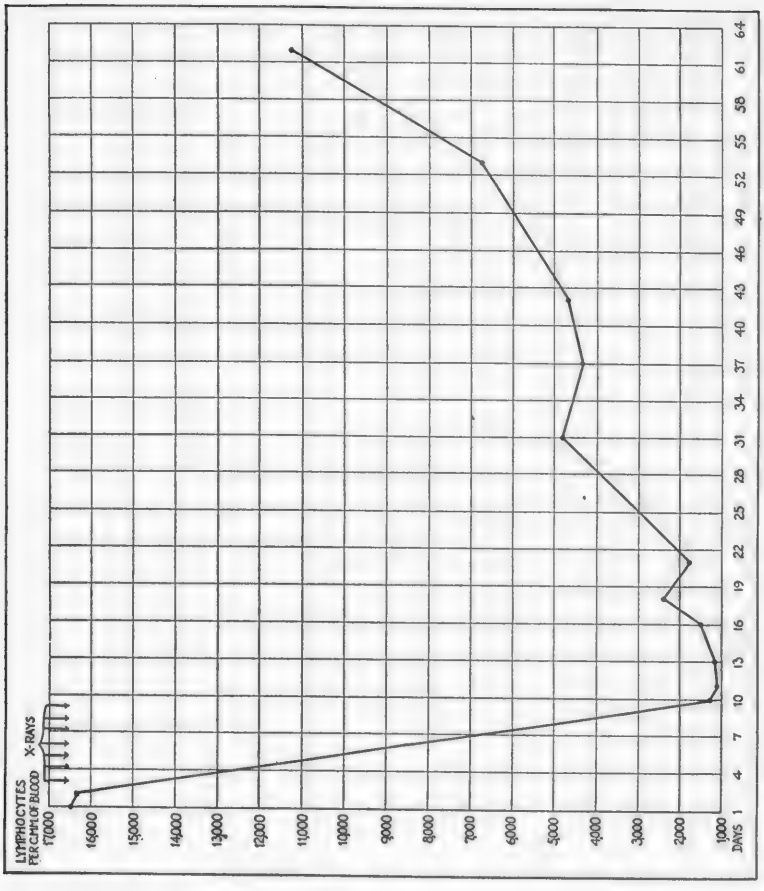
TABLE I.

Pony Receiving 160 Holzknecht Units.

Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
		Per cent.	Total No.	Per cent.	Total No.
	days				
1		43.7	5,790	55.7	7,380
3		48.3	6,859	48.3	6,859
6		44.2	6,906	50.0	7,813
7	1	6.3	1,473	93.7	21,902
8	2	1.2	321	92.7	24,797
9	3	11.7	2,404	84.7	17,406
10	4	24.2	1,785	66.7	4,918
11	5	29.3	1,282	60.3	2,638
12	6	27.0	1,593	66.3	3,912
13	7	31.6	3,410	64.0	6,912

Experiment 3.—A full grown cat was given two treatments in a single day with unfiltered x-rays emitted by a Coolidge tube. One exposure was made on the dorsal and one on the ventral surface of the body. The factors at each exposure were: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 1 minute. The dose, in this instance, was for each exposure $1\frac{1}{2}$, or a total of 3 Holzknecht units. Text-fig. 1, *b* is a curve showing the total number of lymphocytes plotted against the days on which blood counts were made. Table III gives percentages and actual numbers of white blood cells on the days recorded in Text-fig. 1, *b*.

Experiment 4.—A guinea pig was exposed to unfiltered x-rays on 3 successive days, after an initial blood count had been made. On the 5th day, 48 hours after the last x-ray treatment, a second count was made; Text-fig. 1, *c* shows



TEXT-FIG. 2. The effect of x-ray treatment on the circulating lymphocytes of a *Macacus rhesus*.

graphically the fall in the total number of lymphocytes per c. mm. of blood following the administration of the x-rays. Table IV gives the actual figures determined at the two blood examinations. The factors at each treatment were:

TABLE II.

Monkey Receiving 42 Holzknecht Units.

Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
		Per cent.	Total No.	Per cent.	Total No.
	days				
1		62.7	16,537	36.0	9,495
2		56.7	16,415	40.7	11,783
10	1	4.0	1,283	94.7	30,375
11	2	4.7	1,128	95.0	22,800
13	4	7.0	1,155	92.7	15,296
16	7	5.7	1,526	94.0	25,169
18	9	11.7	2,463	88.0	18,524
21	12	7.0	1,787	92.3	23,560
28	19	27.0	4,846	71.0	12,745
37	28	23.3	4,351	74.7	13,950
42	33	31.7	4,771	64.3	9,677
53	44	29.0	6,779	66.7	15,598
63	54	55.7	11,224	40.7	8,201

TABLE III.

Cat Receiving 3 Holzknecht Units.

Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
		Per cent.	Total No.	Per cent.	Total No.
	days				
1		35.0	7,389	55.7	11,759
2		34.7	7,825	56.0	12,628
4	2	5.0	844	94.0	15,876
5	3	8.7	1,005	83.7	9,672
7	5	16.0	3,093	77.0	14,886
8	6	12.0	2,907	79.0	19,144
10	8	11.0	5,194	88.0	41,555*

* Distemper developed later in this cat and the high polymorphonuclear leucocyte count may be due to this.

spark-gap $2\frac{1}{4}$ inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time 3 minutes. This dose represents, approximately, 3 Holzknecht units.

In two experiments on mice a gas tube had been used to generate x-rays. The dose was necessarily indefinite and the only measure of the comparative amount of the x-rays received by each animal consists in the constant established by the fact that the mice included in each experiment were exposed simultaneously and for the same length of time. These experiments are included because they demonstrated the tendency of the circulating lymphocytes to decrease in number after animals had been exposed to the x-rays generated by gas tubes and because this decrease was, in many ways, similar to that observed in Experiments 1 to 4. Furthermore, there is a definite relation between the response of the various animals in a series, as determined by blood counts, to x-ray treatment.

TABLE IV.

Guinea Pig Receiving Approximately 3 Holzknecht Units.

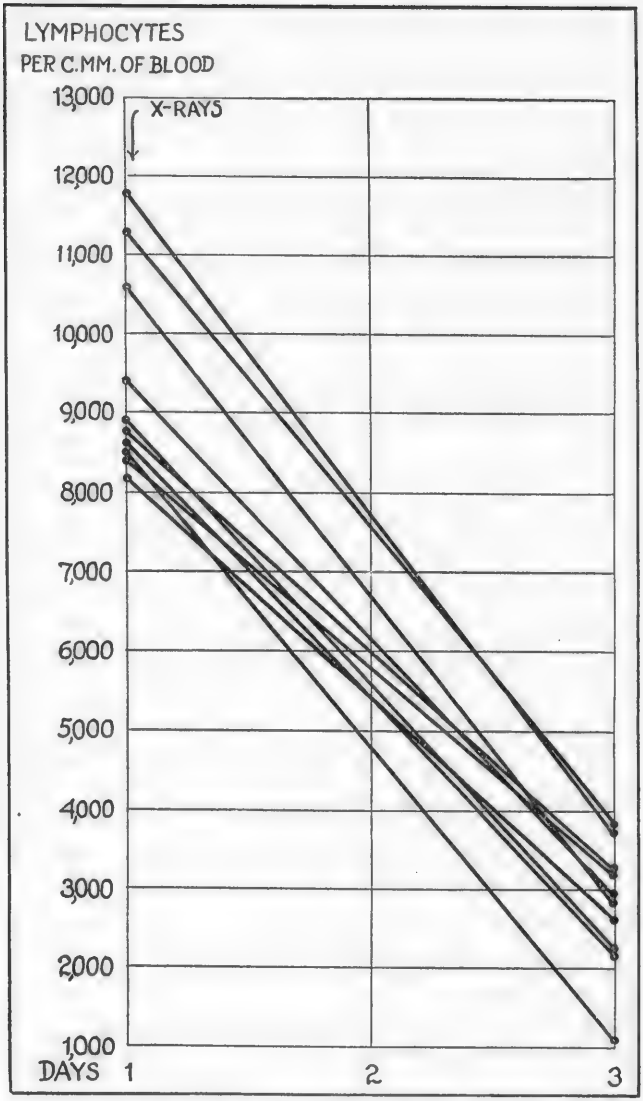
Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
		Per cent.	Total No.	Per cent.	Total No.
	days				
1		36.7	10,606	61.7	17,831
5	2	16.3	4,283	81.3	21,138

Experiment 5.—Ten normal, adult, white rats were simultaneously exposed for the same length of time to unfiltered x-rays generated by a rather soft gas tube. Total white and differential blood counts were made on each animal immediately before and 48 hours after exposure. The effect of the x-rays on the circulating lymphocytes (total number per c. mm.) is shown in Text-fig. 3, where each line represents the difference, in a single rat, between the number of lymphocytes before and after treatment.

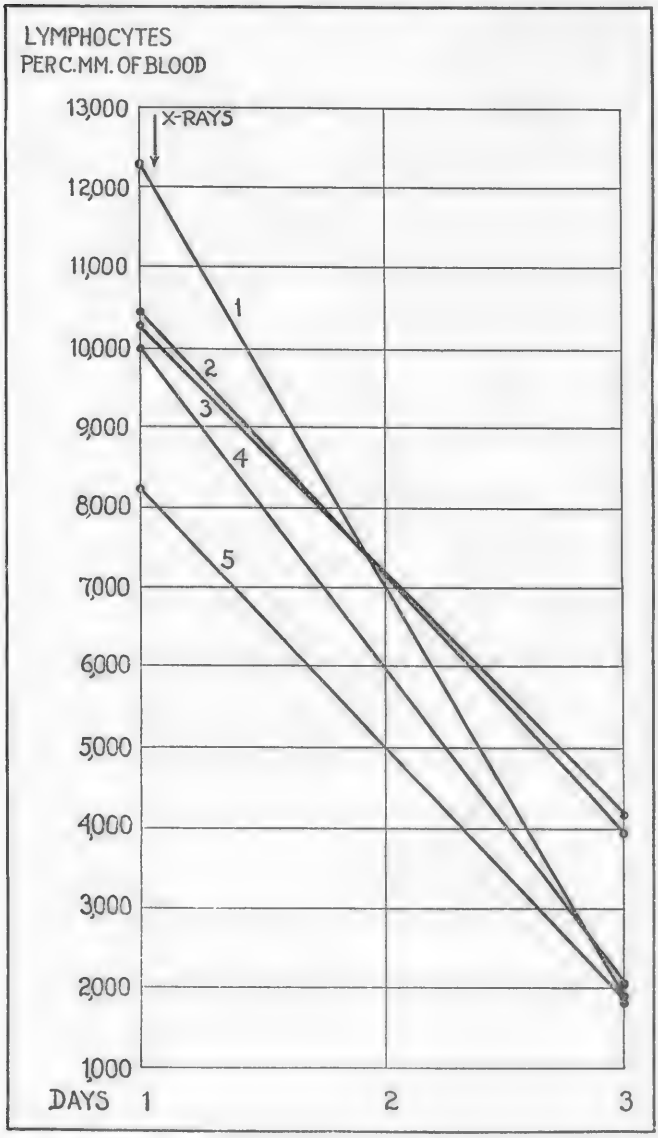
Experiment 6.—The procedure followed in Experiment 5 was repeated with five additional white mice and the results are shown in Text-fig. 4, in which the lines have the same significance as those in Text-fig. 3.

Experiment 7.—Four mice were simultaneously given seven treatments with unfiltered x-rays, the Coolidge tube being used. Blood counts were made on each animal before the first and 48 hours after the last x-ray exposure. The effect of the x-rays on the blood of each mouse is shown in the usual manner in Text-fig. 5; additional data of interest are given in Table V.[15] The factors at

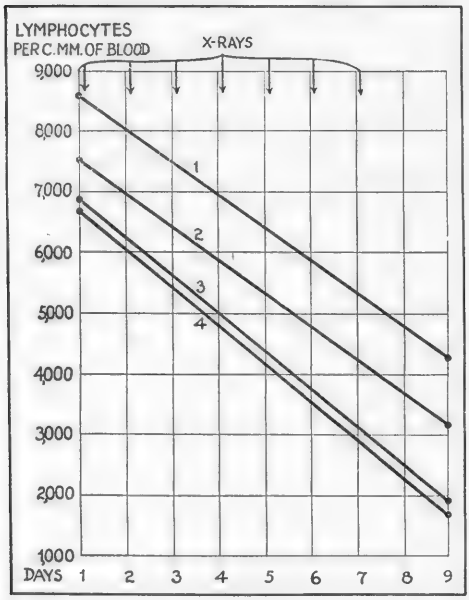
[15] The number of the animal in the curve and in the table corresponds, in this and the following experiments.



TEXT-FIG. 3. The effect of x-ray treatment on the circulating lymphocytes of ten white rats. In this and the following text-figures each line represents the difference in a single animal between the number of lymphocytes before and after treatment.



TEXT-FIG. 4. The effect of x-ray treatment on the circulating lymphocytes of five white mice.



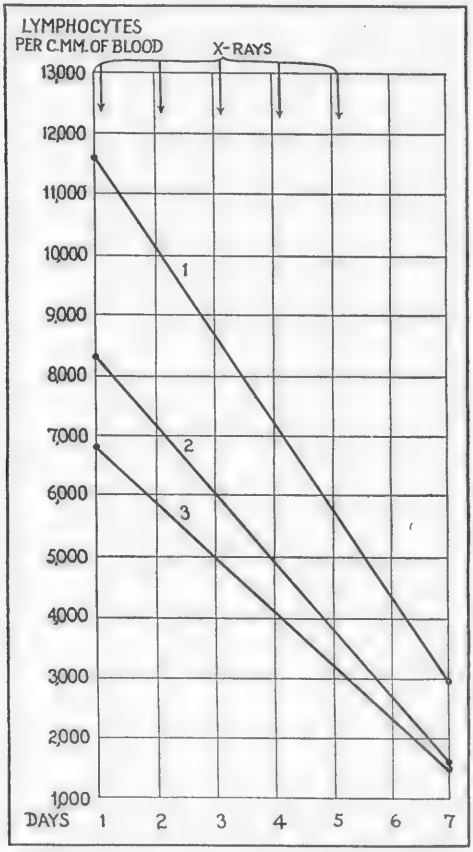
TEXT-FIG. 5. The effect of x-ray treatment on the circulating lymphocytes of four white mice.

TABLE V.

Four Mice Receiving 14 Holzknecht Units.

Mouse No.	Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
			Per cent.	Total No.	Per cent.	Total No.
		days				
1	1		73.0	8,588	27.0	3,180
	9	2	55.3	4,301	44.7	3,477
2	1		90.3	7,527	9.6	800
	9	2	57.0	3,103	43.0	2,341
3	1		48.2	6,909	51.7	7,410
	9	2	42.0	1,914	58.0	2,643
4	1		51.0	6,687	49.0	6,424
	9	2	14.0	1,680	86.0	10,320

each exposure were: spark-gap 2¼ inches, milliamperes 10, distance from the target to the skin 12 inches, and time of exposure 2 minutes. This represents 2, at a single exposure, or a total of 14 Holzknecht units.



TEXT-FIG. 6. The effect of x-ray treatment on the circulating lymphocytes of three adult white rats.

Experiment 8.—Three adult white rats were exposed to the unfiltered x-rays generated by a Coolidge tube. The factors were: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 4 minutes. This dose was repeated daily for 5 days. Each exposure represents 6, a total of

30 Holzknecht units being therefore given. White and differential blood counts made before the first and 48 hours after the last exposure are summarized in Table VI and total lymphocytes per c. mm. of blood at each determination charted in Text-fig. 6.

Experiment 9.—Three rabbits, after preliminary blood counts, were given seven daily, unfiltered doses of x-rays, each dose depending on the following factors: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 4 minutes. This represents 6 at a single dose, or a total of 42 Holzknecht units. The dorsal and ventral surfaces of the animals were exposed to the rays on alternate days. Text-fig. 7 and Table VII refer to the blood counts on these animals.

Experiment 10.—Two rabbits were each given a single treatment with the unfiltered x-rays emitted by a Coolidge tube, which was controlled by the following factors: spark-gap 3 inches, milliamperes 20, distance from the target to the

TABLE VI.

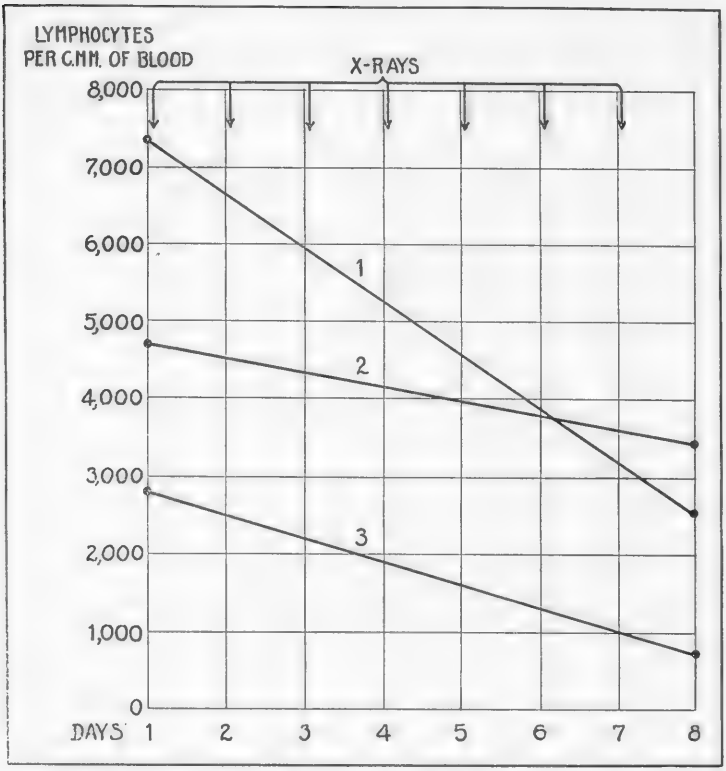
Three Rats Receiving 30 Holzknecht Units.

Rat No.	Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
			Per cent.	Total No.	Per cent.	Total No.
		days				
1	1		89.7	11,683	10.3	1,342
	7	2	62.3	2,990	37.3	1,790
2	1		63.0	8,096	37.0	4,755
	7	2	16.3	1,593	83.7	8,182
3	1		79.2	6,831	20.7	1,785
	7	2	48.7	1,583	51.3	1,667

skin 12 inches, and time 4 minutes. This dose represents 11 Holzknecht units. Text-fig. 8 and Table VIII refer to the blood counts on these animals.

Experiment 11.—Two rabbits at a single exposure were treated with x-rays filtered through 3 mm. of aluminum, the dose approximating 5 Holzknecht units. This was determined by the following factors: spark-gap 9 inches, milliamperes 5, distance from the target to the skin 12 inches, and time 5 minutes and 20 seconds. Text-fig. 9 and Table IX refer to the circulating lymphocytes and polymorphonuclear leucocytes in these animals, before and 48 hours after exposure.

Experiment 12.—Three monkeys were each exposed to the unfiltered x-rays generated by a Coolidge tube for a total of seven treatments. Monkeys 1 and 2 had daily exposures, while Monkey 3 was given the seven treatments in 4 days. The factors at each treatment were: spark-gap 3 inches, milliamperes 10, distance from the target to the skin 12 inches, and time 4 minutes. At each exposure 6, or a total of 42 Holzknecht units were therefore given to each animal. Text-fig. 10 and Table X give the data for these monkeys.

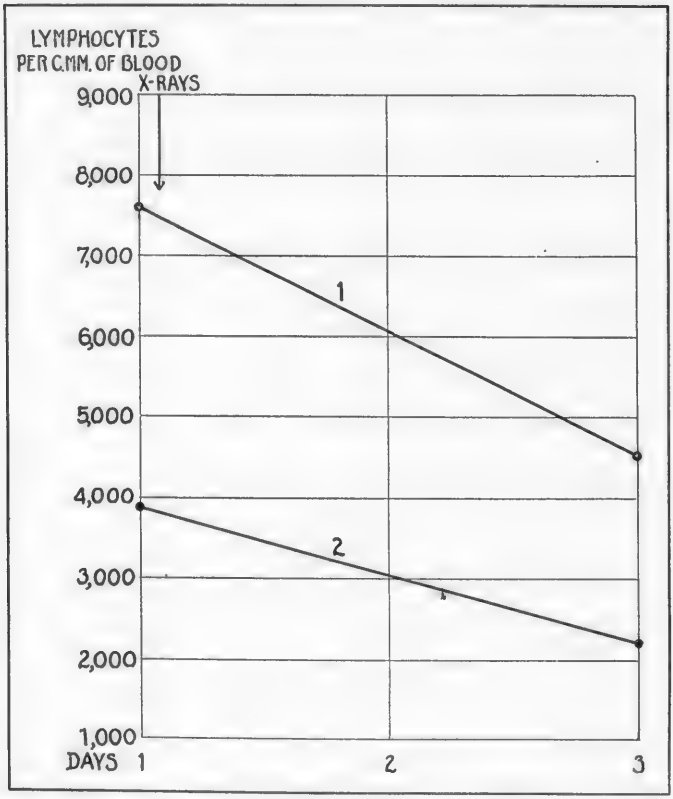


TEXT-FIG. 7. The effect of x-ray treatment on the circulating lymphocytes of three rabbits.

TABLE VII.

Three Rabbits Receiving 42 Holzknecht Units.

Rabbit No.	Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
			Per cent.	Total No.	Per cent.	Total No.
		days				
1	1		55.3	7,383	42.0	5,607
	7	2	54.3	2,570	39.3	1,870
2	1		44.7	4,716	47.7	5,032
	7	2	39.7	3,462	55.7	4,857
3	1		29.7	2,866	64.0	6,176
	7	2	24.3	758	66.7	2,081

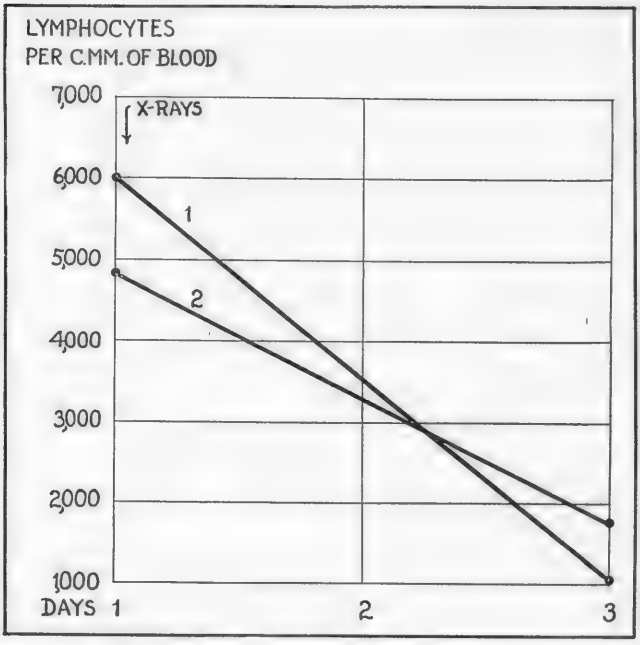


TEXT-FIG. 8. The effect of x-ray treatment on the circulating lymphocytes of two rabbits.

TABLE VIII.

Two Rabbits Receiving 11 Holzknecht Units.

Rabbit No.	Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
			Per cent.	Total No.	Per cent.	Total No.
		days				
1	1		66.3	7,591	31.3	3,584
	3	2	54.7	4,568	41.3	3,449
2	1		51.2	3,878	48.0	3,636
	3	2	32.3	2,196	65.6	4,461

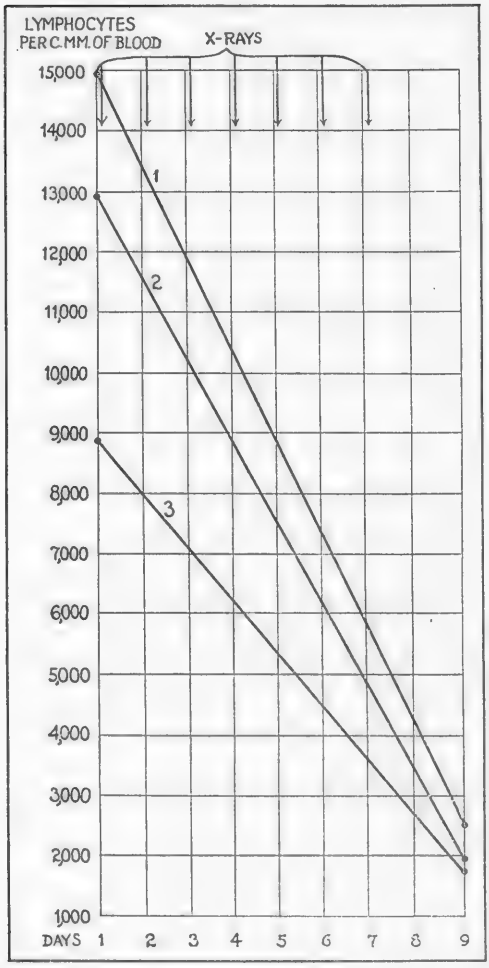


TEXT-FIG. 9. The effect of x-ray treatment on the circulating lymphocytes of two rabbits. The x-rays were filtered through 3 mm. of aluminum.

TABLE IX.

Two Rabbits Receiving Approximately 5 Holzknecht Units (Filtered).

Rabbit No.	Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
			Per cent.	Total No.	Per cent.	Total No.
		days				
1	1		87.3	6,002	11.0	756
	3	2	44.7	1,017	50.7	1,153
2	1		54.0	4,860	39.7	3,573
	3	2	27.2	1,761	65.0	4,209



TEXT-FIG. 10. The effect of x-ray treatment on the circulating lymphocytes of three monkeys.

TABLE X.

Three Monkeys Receiving 42 Holzknecht Units.

Monkey No.	Day of experiment.	Length of time after x-rays.	Lymphocytes.		Polymorphonuclear cells.	
			Per cent.	Total No.	Per cent.	Total No.
		days				
1	1		59.7	14,925	34.3	8,575
	9	2	17.7	2,509	77.0	10,915
2	1		54.0	12,960	42.0	10,080
	9	2	15.0	1,980	77.5	10,230
3	1		54.7	8,861	43.7	7,079
	6*	22	17.7	1,744	81.3	8,008

* In this animal the seven treatments were given in 4 days.

DISCUSSION.

The immediate effect of the x-rays, in the dose employed in these experiments, is a sudden decrease in the circulating lymphocytes, evident in every curve and table in the series. The curves all represent total numbers of lymphocytes, small and large varieties combined, per c. mm. of blood. When the lymphocytes are studied in terms of percentage of total white blood cells the results are not so striking, and, while in most instances there is a definite fall in percentage as well as in actual numbers of these cells after x-ray treatment, an occasional instance is encountered where the percentile change is slight or absent. For example, whereas the total lymphocytes of Rabbit 1, Table VII, decreased from 7,383 before to 2,570 after exposure, the corresponding fall in percentage of lymphocytes was small; *i.e.*, from 55.3 to 54.3 per cent. The latter fall is well within the limits of counting error and is therefore negligible. It seems, therefore, that an estimation of the total number of lymphocytes per c. mm. of blood, determined by multiplying the total white count by the combined percentage of large and small lymphocytes, before and after x-ray treatment offers a more accurate indication of the effect on the blood than can be determined by following percentage figures only. This means, in other terms, that while the x-rays, in most instances, affect the lymphocytes selectively, occasional cases occur in which together with the lymphocytes the granular blood cells are also destroyed.

When the total number of circulating lymphocytes is plotted against the time in days a curve is formed which reaches its lowest level 48 hours after the administration of the x-rays (Text-figs. 1, *a*, *b*, and 2). Following this fall there is a primary rise, which reaches its acme from 3 to 5 days after the last x-ray exposure (3 days in Experiment 1, 9 days in Experiment 2, and 4 days in Experiment 3). A secondary fall then occurs, which reaches its lowest level from 5 to 12 days after treatment, and this is followed by a secondary and, as far as has been determined, permanent rise, which persists for at least 54 days after x-ray treatment (Text-fig. 2). The slight variation in the time relations of the various phases of the curves, shown in Text-figs. 1, *a*, *b*, and 2 are probably due to the fact that in Experiments 1 and 3 the entire dose of x-rays was given in a single day, while in Experiment 2 the seven doses were dispersed over an interval of 8 days. A comparison of Text-figs. 1, *a* and 2 shows that the primary rise occurred on the 3rd and on the 9th day respectively, while the secondary fall reached its lowest limit on the 5th and on the 21st day. It seems possible that the destructive action of the x-rays on the lymphocytes is first felt by these cells in the general circulation and in the spleen (this corresponds to Warthin's view[5]), and that this accounts for the primary fall. In the meantime the lymphogenic cells of the other organs, lymph glands, bone marrow, etc., contribute cells to the blood, these being responsible for the secondary transient rise. When all the lymphogenic tissues have been affected by the x-rays the secondary fall in these cells is apparent in the blood, but it does not reach the extremely low level characteristic of the primary fall, because of beginning regeneration in the spleen. Regeneration of all the lymphogenic tissues later contributes to the permanent rise. It requires a considerable period of time before the lymphocyte-forming tissues regenerate entirely. In Experiment 2 for instance, the tissues are not contributing the normal numbers of lymphocytes to the circulation 54 days after the last x-ray treatment. The curve representing blood lymphocytes (Text-fig. 2) can be seen to be rapidly approaching the pre-treatment level at this time, however, and probably, had the observations been prolonged over a sufficiently long period of time, the lymphocytic elements would again have been present in the blood in normal numbers. When the x-ray treatments are distributed

over a number of days the issues are somewhat confused, and because of overlapping of phases the curves are not so sharply defined as when the entire dose is given in a single day. The primary fall in circulating lymphocytes, which in the case of the animals given x-rays in several doses is really the result of a series of primary falls, one following each treatment, is complete after 48 hours. However, the other phases, as may be seen from Text-fig. 2, are somewhat lengthened on this curve as contrasted with those given in Text-fig. 1, *a* and *b*.

From 24 to 48 hours after x-ray treatment there is, in most instances, a considerable increase in the polymorphonuclear neutrophilic leucocytes, which is well seen in the figures of Tables I and II. This is not evident in the rabbits followed (Tables VII, VIII, and IX) nor in the rats (Table VI). In mice (Table V) there is a tendency for these cells to exhibit a post-treatment rise, but this is not striking. The blood of the guinea pig showed a moderate increase in these cells after x-ray exposure.

When there is a primary rise in polymorphonuclear leucocytes, it is followed by a primary fall, which reaches its lowest level at a time when the lymphocytes are beginning to rise, the 6th day after x-ray exposure in Experiment 1, and the 3rd day in Experiment 2 (Tables I and II). Later there is a gradual rise to normal, about which level the number of neutrophilic cells fluctuates within wide limits. This is well seen in Table II. The polymorphonuclear cells are much less affected than the lymphocytes even when large doses of x-rays are given, and after the primary stimulation and later depression the return to the normal number per c. mm. occurs at a time when the lymphocytes are still at a very low level (Tables I and II). This seems to confirm Warthin's contention[5] that the bone marrow is affected somewhat later and to a more limited extent than the lymphogenic tissues proper. Circulating eosinophils and basophils, as well as large mononuclear and transitional leucocytes, usually share in the stimulations and depressions of the neutrophilic cells. This is what would be expected, as they all belong to the granular series and all originate in the bone marrow.

A glance at the curves shown in Text-figs. 3 to 10 leaves little to be said regarding the regularity with which the circulating lymphocytes of a series of animals of the same species respond to the same

dose of x-rays. Exceptions to the general rule of a quantitative relation between the reactions of the several animals of a series are uncommon. Apparently Rabbit 2 in Text-fig. 7 and Rabbits 1 and 2 in Text-fig. 9 belong in this category. The latter animals received the only treatment with a filtered dose of x-rays in the entire group reported, and it is not possible to draw conclusions from this single instance. The lymphocytic fall is evident, however, in both animals. Rabbit 2 in Text-fig. 7, then, is the only exception encountered and no explanation is apparent. There is a decrease in the circulating lymphocytes of this animal, following treatment with the x-rays, but this is not so great as in the companion rabbits, Nos. 1 and 3. Exceptions are naturally encountered in all biological experiments, and as this is the only striking instance found in the entire study it seems safe to disregard it and to accept the other results as manifesting accordance to a general law, inasmuch as they are regular and constant.

In Experiment 9 x-rays equivalent to 42 Holzknecht units destroyed, if all the rabbits are included, an average of 55.1 per cent, or, if Rabbit 2 is disregarded and the others (Nos. 1 and 3) with comparable lymphocyte curves are considered, an average of 69.4 per cent of the total number of circulating lymphocytes in the 48 hour interval following treatment. In the monkeys, given the same dose, Experiment 12, Table X, the effect of the x-rays was much more pronounced, 82.7 per cent of the circulating lymphocytes having disappeared 48 hours after treatment. It would seem that the monkey is more susceptible to the x-rays, in as far as the lymphocytic reaction represents an accurate measurement of the degree of susceptibility, than the rabbit. In the pony a dose of 160 Holzknecht units destroyed 95.4 per cent of the circulating lymphocytes, as determined 48 hours after treatment. In the monkey, Experiment 2, Text-fig. 2, Table II, 42 Holzknecht units were sufficient to destroy 93.1 per cent of the lymphocytes of the blood. This contrasts sharply with the three monkeys in Experiment 12, Text-fig. 10, Table X, in which the same dose destroyed an average of 82.7 per cent, and in the animal most affected, No. 3, but 84.7 per cent.

The lymphogenic tissues of the cat seem to be more susceptible than those of any other animal studied, inasmuch as 3 Holzknecht

units were sufficient to destroy 89.2 per cent of the circulating lymphocytes. In some of the experiments it was not possible to determine accurately the doses of x-rays given, but from the information at hand it seems that different species of animals vary considerably in their response to a given dose of x-rays. Those studied here would seem to follow a series, progressing from the most to the least susceptible, somewhat as follows: cat, monkey, guinea pig, rabbit, rat, mouse, and pony.

SUMMARY.

1. X-rays in large doses affect the lymphocytes before any of the other circulating cells.

2. There is a sharp fall in the total number of circulating lymphocytes, which is complete 48 hours after x-ray treatment.

3. Following the immediate decrease in the circulating lymphocytes there is a primary rise, followed by another fall, which in turn is followed by a permanent rise of these cells to normal.

4. The effect of the x-rays on different species of animals varies considerably, but in those studied, cat, monkey, guinea pig, rabbit, rat, mouse, and pony, the selective action on the lymphocytes was in all instances apparent.

5. When several animals of the same species are given the same dose of x-rays, the effect on the circulating lymphocytes seems to be quantitatively parallel, when determined by blood counts.

6. The polymorphonuclear neutrophilic leucocytes, when affected at all, increase in number immediately after the administration of the x-rays and then tend to decrease below their normal level. This decrease is followed by a return to normal many days before the lymphocytes reach their original level.

7. The other cells of the blood follow the neutrophilic curve.

8. Percentage figures, as determined by differential blood counts, do not give an accurate indication of the effect of the x-rays. It is only when these are multiplied by the total white blood count that a figure, representing the total number of cells of the series per c. mm. of blood, is obtained, which varies to the stimulus in a constant manner, the variations being practically quantitative.

STUDIES ON X-RAY EFFECTS.

II. Stimulative Action on the Lymphocytes.*

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Taylor, Witherbee, and Murphy[1] have reported on the destructive action of x-rays on the circulating lymphocytes, confirming and extending the earlier work on this subject. It was noted by Murphy in his studies on x-ray effects[2] that while large doses destroyed, a small dose of x-rays would bring about a stimulation of the lymphocytes. This observation was later applied experimentally.[3] In the earlier experiments the older type of x-ray tube was used, and it was practically impossible to establish a standard and uniform dose. With the introduction of the Coolidge tube the difficulty was eliminated to a large extent, and there was an opportunity to check this observation and extend it.

Mice have not been used here as in the previous experiments for the reason that blood counts could not be made on these animals more frequently than once a week without causing too marked a fluctuation.

EXPERIMENTAL.

Brown rabbits of the same relative size were used in the nine experiments. All the animals were kept in separate cages. Several blood counts were made on these normal rabbits, and they were then exposed to the rays of a Coolidge tube. A dose of low penetration

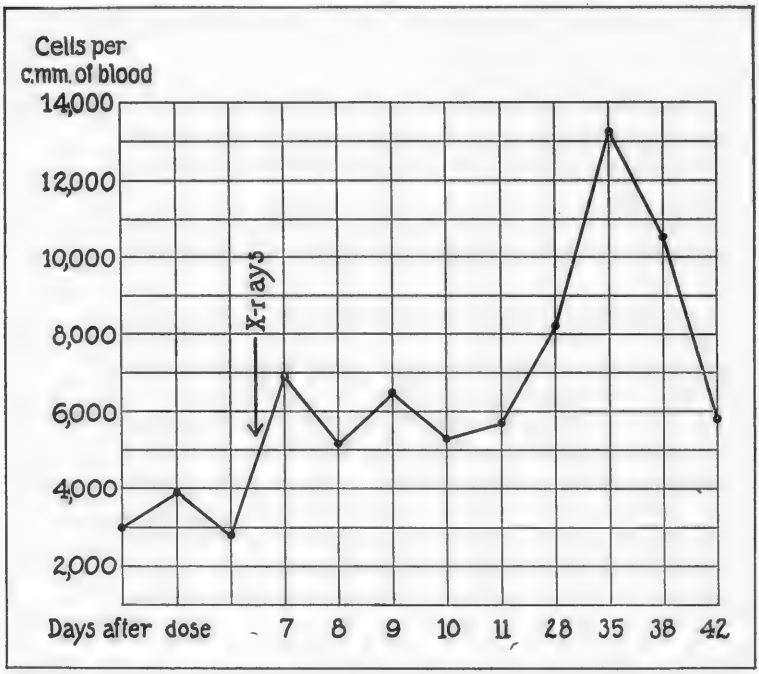
*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

[2] Murphy, Jas. B., unpublished observation.

[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

was applied to the dorsal area: the spark-gap measured $\frac{7}{8}$ inch, the milliamperage was 25, the distance from the target to the back 8 inches, and the time of exposure 20 minutes. The temperature 8 inches from the target was 31°C. In almost every case a blood count made 48 hours after exposure showed a slight drop in the lymphocytes.

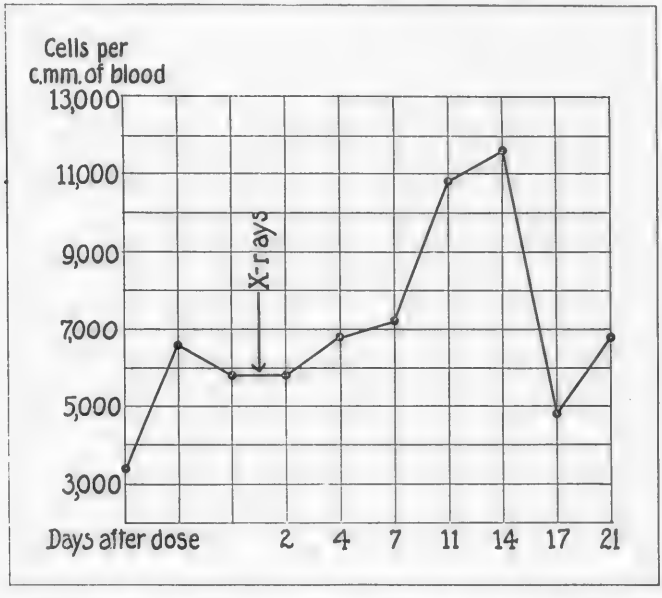


TEXT-FIG. 1. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

Experiment 1 (*Text-Fig. 1*).—Three consecutive counts were made, the lymphocytes numbering 3,000, 4,000, and 2,800 respectively, and the rabbit was then exposed to x-rays. The count made 1 week later showed a stimulation in the cells, the number of lymphocytes being 6,900. Counts made at intervals showed slight fluctuation until the 28th day, when the count reached 8,300. On the 35th day there were 13,300 absolute lymphocytes. In the course of the 7 days following the last count the number of cells fell to 5,800.

Experiment 2 (*Text-Fig. 2*).—Considerable fluctuation was shown in the normal counts. The first count 48 hours after exposure to x-rays showed little or no change, and for 7 days afterwards there was little increase, the lymphocytes numbering 5,000, 6,000, and 7,000 respectively, but on the 11th day the number had risen to 10,500, and on the 14th day to 11,600. They fell to 4,800 on the 17th day and in the last count rose to 6,900.

Experiment 3.—The standardizing counts were 6,900, 6,370, and 7,000. 48 hours after exposure to the x-rays a slight fall was noted, the number of cells



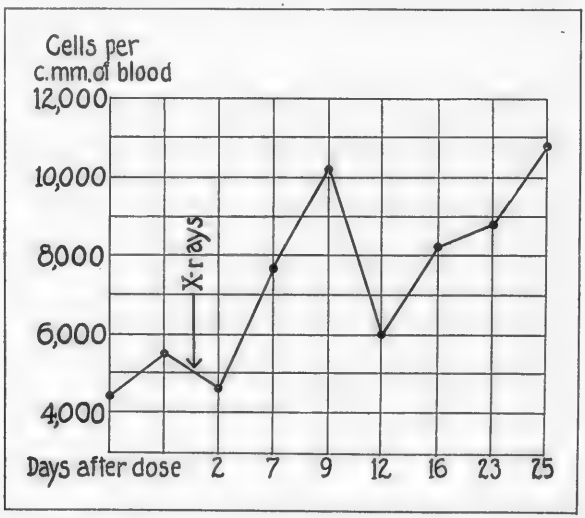
TEXT-FIG. 2. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

at that time being 5,100. On the 4th day there were 5,300 lymphocytes, and 6 days afterwards 10,240, showing marked stimulation. On the 8th day the number had decreased to 6,100 and on the 15th day to 4,000. After that there was a slight rise (6,500) on the 18th day and also on the 23rd, followed by another fall to 3,410. A rise to 8,200 was noted on the 64th day, and the last count, at which the number of cells was 6,300, was made on the 70th day after exposure.

Experiment 4.—4,300, 4,670, and 4,900 were the lymphocyte counts of a rabbit before x-ray treatment. 48 hours after exposure there was a slight increase (6,600). The 4th day the number of cells was 4,500, the 6th day 6,500, and the

15th day, when the last count was made, 5,979. This rabbit did not show a stimulation as did those of the preceding experiments.

Experiment 5 (*Text-Fig. 3*).—At the two counts preceding x-ray treatment there were 4,560 and 5,300 lymphocytes respectively. 48 hours after exposure to the x-rays there was a slight fall to 4,600, and on the 7th day a rise (7,600) which continued to the 9th day and at that time reached 10,200. On the 12th day there was a slight decrease, but from that time to the 25th day, on which the last count was made, there was a continued rise in the number of lymphocytes, which finally reached 10,900.



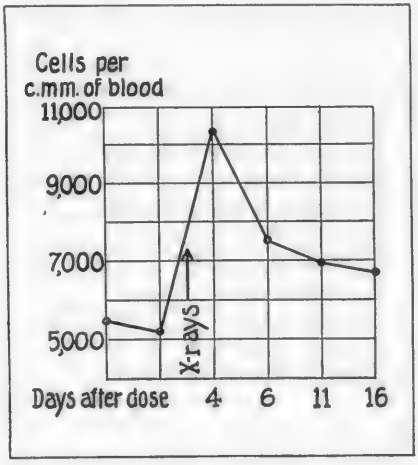
TEXT-FIG. 3. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

Experiment 6.—The normal counts showed a great fluctuation, the lymphocytes numbering 3,500 at the first count, at the second 6,100, and at the third 6,290. 48 hours after x-ray exposure they fell to 3,600, rose on the 4th day to 6,400, and fell again on the 6th day to 3,800. The cell counts, which were made at intervals until the 25th day after exposure, continued to rise and fall, never reaching a point beyond 5,900 or below 3,000.

Experiment 7.—The normal counts in this instance were 4,900 and 5,800. 48 hours after exposure to the x-rays the cells numbered 5,600, the 4th day 5,400, the 6th 5,700, and the 8th 6,180, showing a slight increase. On the 13th day the number of cells was 5,600, and on the 20th day, when the last count was made, 5,750.

Experiment 8.—2,270, 5,340, and 2,267 were the three consecutive lymphocyte counts made before x-ray treatment on a rabbit. 48 hours after treatment there was little or no change, the cells numbering 2,200. On the 4th day there was a rise to 4,800, on the 6th to 7,900. 11 days after exposure the number was 6,560. Subsequently there was a decline to 3,900 (13th day) and finally, on the 25th day, to 3,000.

Experiment 9 (*Text-Fig. 4*).—Two normal counts, the first 5,500, the second 5,100, were followed by the short x-ray dose. The 48 hour count was not made on this rabbit. The first count following exposure was made 4 days afterwards, the lymphocytes then numbering 10,390. On the 6th day there was a fall to 7,500, on the 11th day to 6,900, and on the 16th day to 6,700.

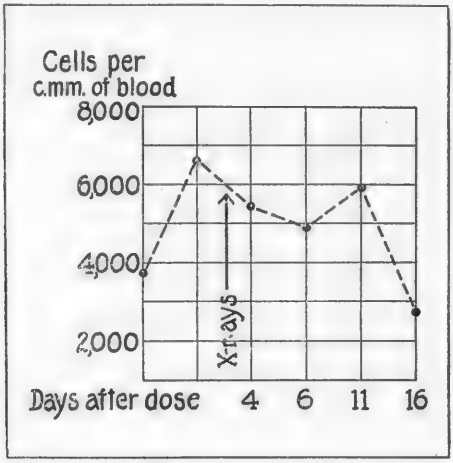


TEXT-FIG. 4. Effect of unfiltered x-rays of low penetration on the lymphocytes of a rabbit.

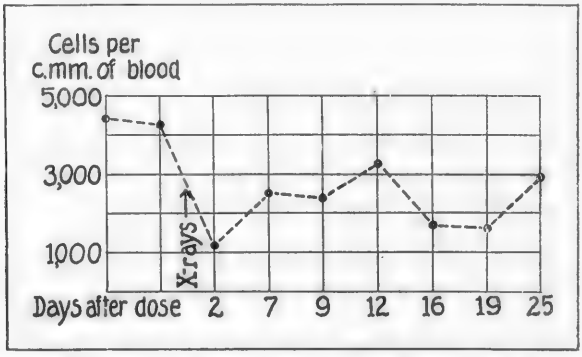
A comparable dose of filtered x-rays[4] was used also on a smaller number of brown rabbits (spark-gap 6 inches, milliamperage 5, distance from the target to the back 10 inches, time 26 minutes and 57 seconds). The rays were filtered through 3 mm. of aluminum. The animals were exposed in the same way (over the dorsal area) and kept under the same conditions as those of the preceding experiments.

[4] Remer, J., and Witherbee, W. D., *Am. J. Roentgen.*, 1917, iv, 303.

Experiment 1, a (*Text-Fig. 5*).—In the two counts preceding x-ray exposure the number of lymphocytes was not very constant, being 3,700 at the first and 6,600 at the second. After the filtered x-ray dose (6 inch spark-gap, milliamper-



TEXT-FIG 5. Effect of filtered x-rays on the lymphocytes of a rabbit.



TEXT-FIG. 6. Effect of filtered x-rays on the lymphocytes of a rabbit.

age 5, distance from the target to the back 10 inches, and time 26 minutes and 57 seconds) the count showed little change, 5,470 in the first count, which was made on the 4th day, 4,900 on the 6th day, 5,900 on the 11th day, and 2,733 on the 16th day.

Experiment 2, a (Text-Fig. 6).—The lymphocytes numbered 4,400 and 4,350 at the two counts preceding x-ray treatment. 48 hours after exposure there was a marked fall (1,230), and on the 7th day a slight rise (2,500). On the 9th day the count was similar to that of the 7th; on the 12th it was 3,500, on the 16th 1,700, on the 19th 1,720, and on the 25th 2,981. There is no sign of stimulation in Experiment 2, *a*.

DISCUSSION.

It is of interest to note in these experiments that the x-ray dose used was of low penetration, the spark-gap being under an inch. The use of a larger spark-gap with apparently the same dose of x-rays did not give a stimulation. This suggests that the effect on the lymphoid organs is not the result of a direct action of the rays but is secondary to changes brought about either in the circulating blood or in the superficial tissues. The amount of x-rays penetrating to the deeper structures with this dose must be infinitesimal.

Another question arises as to the nature of the energy generated by the x-ray tube operated on so small a spark-gap. This point has not yet been taken up, but it is conceivable that other factors than the pure x-rays may play a part.

The results obtained in this small series of animals would not in themselves be accepted as conclusive evidence but are of interest principally as a parallel to our histological studies.[5] It is conceivable that a marked stimulation may be taking place in the lymphoid organs without a proportionate number of these cells being thrown into the circulation. The question in itself offers an interesting problem of just what determines the number of cells in the circulation. It is well known that individuals with normal counts react differently in the number of cells thrown into the circulation in response to infections. So here, even with marked stimulation taking place in the lymphoid tissue of the glands and spleen, in only a part of the animals perhaps could we expect this stimulation to be evidenced by an increase in the number of lymphocytes in the circulating blood.

[5] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 83.

SUMMARY.

This study consists of blood counts on nine rabbits after an exposure to x-rays of a $\frac{7}{8}$ inch spark-gap, milliamperage 25, distance from the target 8 inches, and time of exposure 20 minutes.

In seven of the nine animals there resulted an increase of the circulating lymphocytes. In five of these the increase was marked and in two others definite but not striking.

Of the two animals which showed no stimulation one showed marked fluctuation of counts both before and after x-rays and the other little or no change.

The higher penetrating dose (6 inch spark-gap, milliamperage 5, distance from the target 10 inches, time 26 minutes and 57 seconds) given to two animals produced no appreciable stimulation.

STUDIES ON X-RAY EFFECTS.

III. Changes in the Lymphoid Organs after Small Doses of X-rays.

By WARO NAKAHARA, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 8 and 9.

(Received for publication, October 21, 1918.)

The observations of previous investigators have emphasized the fact that x-rays are preeminently and selectively destructive to lymphoid cells (Heineke[1]). Thomas, Taylor, and Witherbee[2] have shown that small doses of x-rays will bring about a stimulation of the circulating lymphocytes, confirming the earlier observations made in this laboratory.[3] As in the experiments on the effect of heat on the circulating lymphocytes,[4] there was a fall immediately after exposure, followed by a more or less gradual rise.

The histologic findings in the lymphoid tissues of mice treated with heat have already been reported,[5] and it has been shown that the lymphocytosis induced by heat is due to the proliferation of certain cells in reaction to the extensive destruction of the tissue by this agent. *A priori*, since x-rays are known to be destructive to lymphoid tissue, the lymphocytic changes observed seemed to be due to a similar cause. The experiments to be reported here were undertaken in order to determine this point.

[1] Heineke, H., *Münch. med. Woch.*, 1903, l, 2258; *ibid.*, 1904, li, 1382; *Mitteil. Grenzgeb. Med. u. Chir.*, 1905, xiv, 21.

[2] Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 75.

[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

[4] Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, xxix, 1.

[5] Nakahara, W., *J. Exp. Med.*, 1919, xxix, 17.

EXPERIMENTAL.

Six normal rabbits of the same color (white), and of approximately the same age, were treated with the same dose of x-rays as was used by Thomas, Taylor, and Witherbee (spark-gap $\frac{7}{8}$ inch, milliamperage 25, distance 8 inches, time 20 minutes). The animals were killed at intervals, and tissues were taken (*a*) immediately, (*b*) 48 hours, (*c*) 4 days, (*d*) 7 days, (*e*) 10 days, and (*f*) 14 days after treatment. This experiment has been duplicated with six brown rabbits. In the second experiment, however, animals were killed two by two (*a*) 24 hours, (*b*) 3 days, and (*c*) 14 days after they were x-rayed, these periods being found to be critical from the result of the first experiment.

For fixation, Carnoy's chloroform-alcohol-acetic (6:3:1) was easy to use and gave good results. Its use saved much time by dispensing with the necessity of passage through the grades of alcohol. Some tissues were fixed in Zenker's fluid for control. The staining was done almost exclusively with Heidenhain's iron-hematoxylin, though a few sections were stained by the ordinary method of hematoxylin and eosin.

Results.

Spleen.—The general histologic condition of the spleen immediately to 24 hours after the treatment is approximately normal. Necrotic cells are extremely scarce in both the nodule and the pulp. The number of mitotic figures in the germinal center of the nodule varies; as a rule, it shows in section a few, sometimes several, and more rarely ten. In the normal spleen of the adult rabbit the germinal centers contain but few mitotic figures, and frequently none. The excessive abundance of mitotic figures after treatment may therefore be taken to indicate the stimulation of these cells.

Sections taken 48 hours after treatment show a condition apparently identical to that just described. The number of necrotic cells in the pulp seems to be slightly larger if anything, but not large enough to be regarded as abnormal.

4 days after the treatment the signs of stimulation become most marked. While the general histologic condition remains unchanged,

the number of mitotic figures in the germinal center of the nodule is decidedly increased.) The germinal center is seen in the section to contain usually several, but sometimes about ten, mitotic figures (Figs. 1 and 2). A comparison of this with the normal condition, in which the proliferative activity in the germinal center is limited, seems to warrant the conclusion that the small dose of x-rays acts as a stimulant to the proliferation of the lymphoid cells.

Sections were taken on the 7th, 10th, and 14th days after treatment. The general histologic condition of all the sections was found to be normal, with the exception of a slightly enhanced mitosis. In the sections taken on the 7th day the number of mitotic figures in the germinal center of the nodule was seen to be smaller, and the tendency to decrease was manifested even more markedly in the sections taken later (on the 10th or 14th day). Even in the later sections, however, the proliferative activity of the cells, judged from the standpoint of mitosis, seemed slightly above the normal.)

Lymph Glands.—In addition to the mesenteric lymph gland, cervical, axillary, and inguinal glands were taken for comparison. The changes in these glands were found to be so nearly uniform in character that a general description will apply to all of them.

The most striking deviation from the normal in the histology of lymph glands immediately after the treatment is the great abundance of mitotic figures in the nodule. The dividing cells are not localized, but are found all over the nodule. Some mitotic figures are seen in the internodular spaces in the cortex and also in the medulla, where, as a rule, very few if any division figures are normally present. The whole tissue was seen to be almost entirely free from degenerating cells. Some evidences of necrosis were, of course, observed here and there, but they were no more conspicuous than those seen in normal tissue.

48 hours after treatment the signs of stimulation of the cells were even more evident than immediately after treatment. All nodules contained excessively large numbers of dividing cells, each of the ordinary sized nodules showing in a section at least ten, and not infrequently as many as twenty mitotic figures (Figs. 3 and 4). The general condition of the gland is similar to that immediately after the treatment, except that there is a slight increase of necrotic cells, mainly in the pulp spaces.

Mitotic figures appear slightly less numerous in the tissue taken 4 days after treatment. The nodule usually shows several mitotic figures in a section. These conditions may still be taken as slightly abnormal. The occurrence of the dividing cells is more or less localized in the nodule, and in many instances they constitute rather typical germinal centers.

In sections taken 7, 10, and 14 days after the treatment, conditions are similar to those just described. The slightly excessive occurrence of mitosis as late as the 14th day is well shown in many nodules in the form of actively proliferating cells of the germinal center.

DISCUSSION.

As far as the results of lymphocyte counts show, the nature of lymphocytosis induced by heat and of that brought about by x-rays is indistinguishable, since the lymphocytic changes are exactly parallel in both cases; *i.e.*, always with a characteristic fall preceding the marked rise. (The idea that the phenomenon of lymphocytosis is of the same nature, regardless of whether the agent used for its production is heat or x-rays, seems probable, especially when we recall the results of Heineke[1] and Warthin,[6] who have shown that the effect of x-rays on lymphoid tissue is, in the main, similar to that of heat, as described by us in another paper.[5]) They used the x-rays, however, in different dosage from ours, and did not, furthermore, make observations during the critical period after the x-ray treatment, when an excessive multiplication of the cells may possibly take place. Notwithstanding the apparent similarity in the nature of the two phenomena which have been pointed out, (the results of the experiments described in the present paper show conclusively that the lymphocytosis induced by the small dose of x-rays is due to the primary stimulative effect of the agent and hence is fundamentally different in nature from the similar lymphocytic change induced by heat, which is a sort of regenerative phenomenon.)

Throughout the course of the experiment no indication has been observed that suggests the injurious effect of the dose upon any of the

[6] Warthin, A. S., *Physician and Surg.*, 1907, xxix, 1.

lymphoid tissues examined. On the other hand, mitotic figures were seen to become gradually more abundant after the treatment. In the spleen this enhanced proliferative activity of the cell reached its height about 4 days after the treatment, and the more or less distinct indications of the stimulation persisted in the germinal center up to the 14th day after the treatment. In the lymph glands the stimulative change is distinctly manifested earlier and is more extensive than in the spleen.

These histologic findings are in harmony with the results of the blood cell counts, which show that the increase of lymphocytes becomes most pronounced about 1 week after the treatment. If the lymphocytosis is due to the stimulation of the lymphoid tissues, the latter should show the change before the former becomes evident, and this is apparently what takes place.

SUMMARY AND CONCLUSION.

1. The small dose of x-rays applied to the rabbit has no appreciable destructive effect on the lymphoid tissue.

2. Indications of stimulation of the lymphoid tissue appear immediately after the treatment, become most pronounced in 2 (in lymph glands) to 4 (in the spleen) days, and persist, in a slight degree, up to the 14th day.

3. These facts suggest that the lymphocytosis induced by the small dose of x-rays is due to a primary stimulative effect upon the lymphoid tissue of the animal.

EXPLANATION OF PLATES.

PLATE 8.

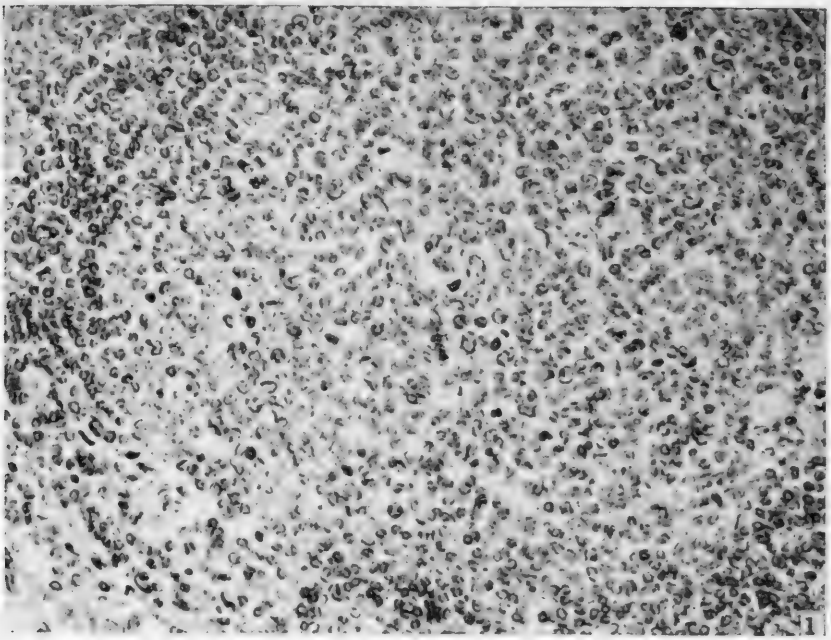
FIG. 1. A splenic nodule, 4 days after the treatment, showing extensive stimulation. × 350.

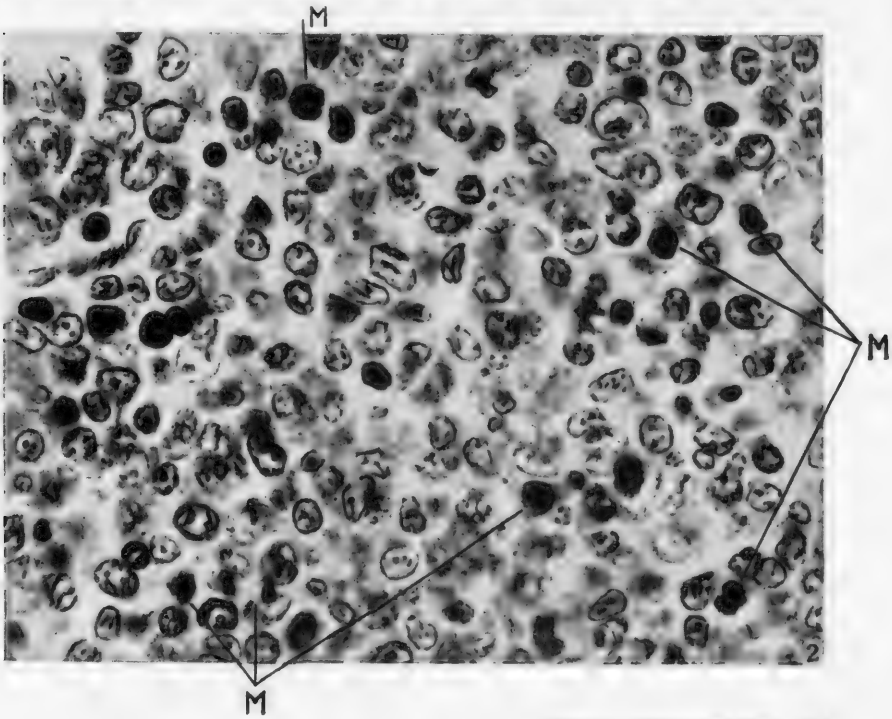
FIG. 2. The same, showing mitotic figures (*M*) in higher magnification. × 1,000.

PLATE 9.

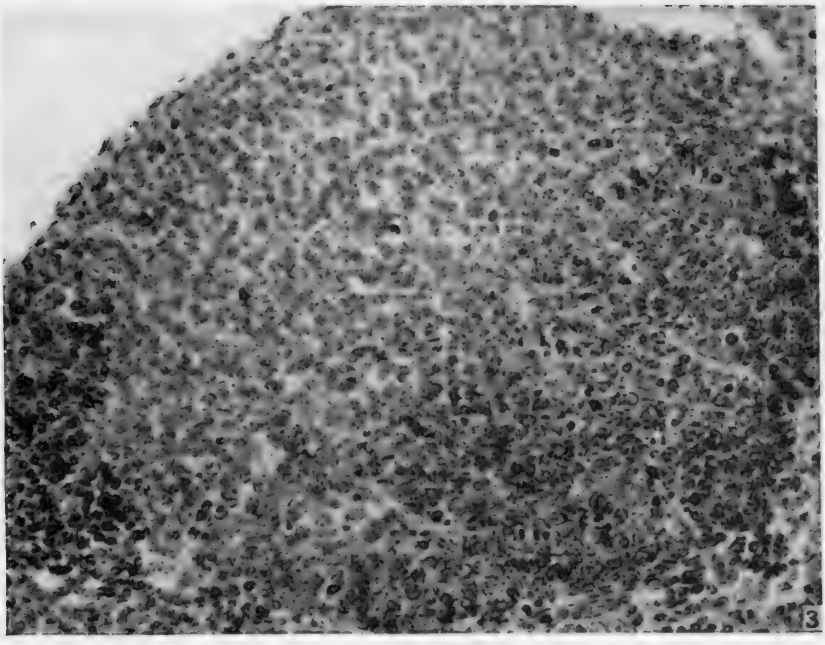
FIG. 3. A nodule of the mesenteric lymph gland, 48 hours after the treatment, showing intense stimulation. × 350.

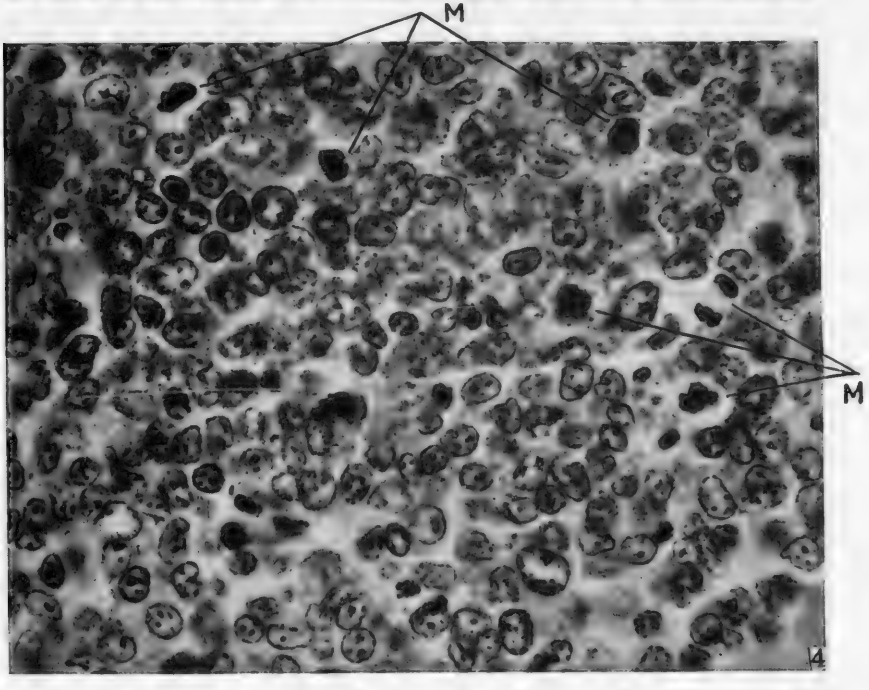
FIG. 4. The same, showing mitotic figures (*M*) in higher magnification. × 1,000.





(Nakahara: Studies on x-ray effects. III.)





(Nakahara: Studies on x-ray effects. III.)

STUDIES ON X-RAY EFFECTS.

IV. Direct Action of X-rays on Transplantable Cancers of Mice.*

By ELSA HILL, JOHN J. MORTON, M.D., and WILLIAM D. WITHERBEE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 21, 1918.)

It has been shown in this laboratory that x-rays administered in doses sufficient to destroy a large proportion of the lymphoid tissue reduce the resistance of the animal to transplanted cancer.[1] Furthermore, both potential and established induced immunity can be destroyed by a similar process.[2] It has also been shown that small doses of x-rays sufficient to stimulate the lymphocytes increase the resistance to cancer.[3] These observations bring up a number of interesting points with regard to x-rays as a therapeutic agent in the treatment of cancer. The literature on this subject is so extensive and contradictory that no attempt will be made to review the previous work. The question of immediate interest to us in this investigation is whether or not x-rays, even in a dose above that possible for therapeutic purposes, will kill the cancer cell. It is necessary in the light of the experiments mentioned above to rule out the action of this agent on the animal itself.

To determine the cumulative, as well as the immediate effect of the direct action of powerful doses of x-rays upon tumor cells, we undertook to grow a Bashford mouse tumor (Adenocarcinoma No. 63) in successive generations of white mice, subjecting the tumor to like doses of x-rays after excision and before inoculation at each transplantation. An actively growing tumor which had been propagated

*This investigation was carried out by means of funds from the Rutherford Donation.

[1] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 204.

[2] Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 1.

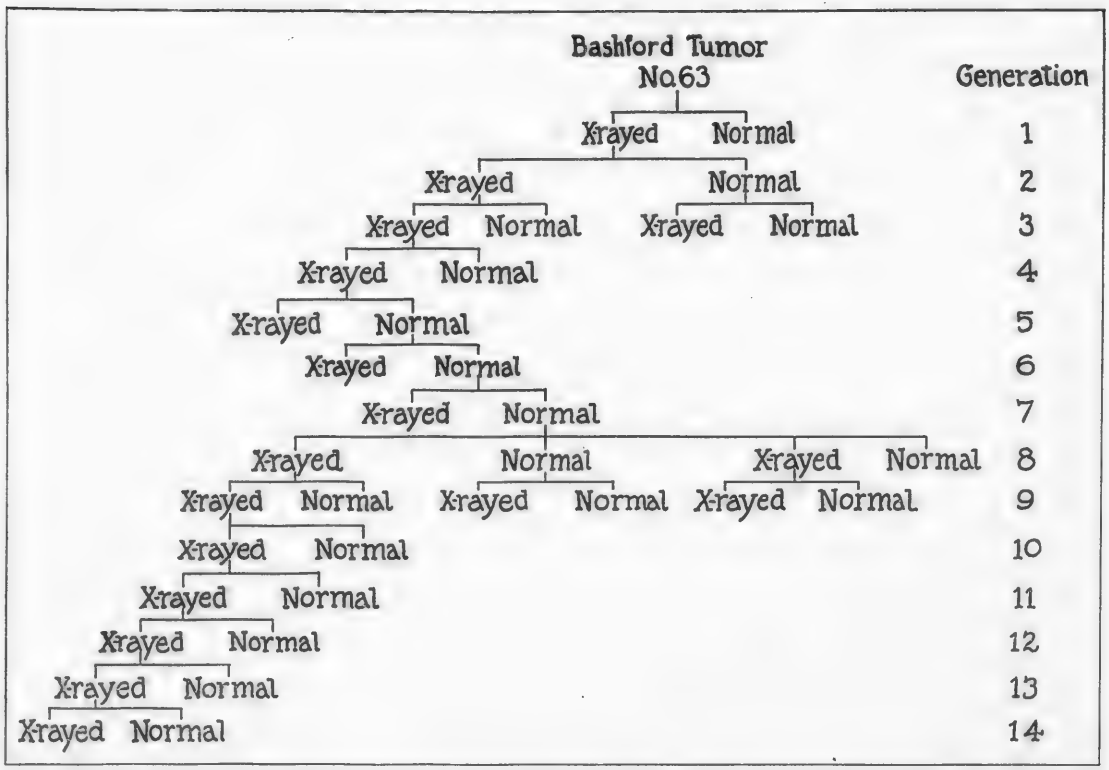
[3] Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, xxii, 800.

in the laboratory for some time was used, and an x-ray dose selected which was purposely above the range of therapeutic dosage: Coolidge tube, spark-gap 8 inches, milliamperes 5, distance 6 inches, and time 2 minutes and 35 seconds. The percentage of takes and rate of growth of the tumor were observed in fourteen generations, extending over a period of 17 months with eleven exposures between transplantations.

Later, considering the possibility of greater absorption of less penetrating rays, we undertook a similar experiment with the following dose: Coolidge tube, spark-gap 1 inch, milliamperes 25, distance 8 inches, and time 20 minutes. In this experiment the tumor was observed in four generations.

Method.

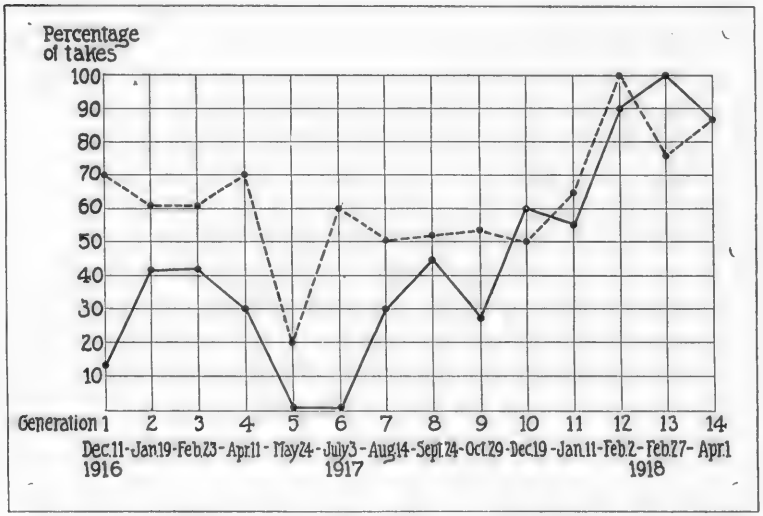
In both experiments (Nos. 1 and 2), the technique used was as follows: Wherever possible a healthy, actively growing tumor, approximately 1.5 by 1.5 cm., was chosen for inoculation. The mouse was killed and the tumor removed under aseptic conditions. It was then cut into halves and each half placed in a small Petri dish, covered with a single layer of sterile gauze, and set into a large Petri dish containing sufficient salt solution to keep the gauze slightly moist. One-half remained in the laboratory at room temperature, while the other half was subjected to x-rays. The temperature at the surface of the gauze did not rise above 33°C. in Experiment 1, or 42°C. in Experiment 2. The x-rayed portion of the tumor was then divided into small pieces of uniform size. Care was taken to select for inoculation the outer actively growing portions of the tumor. Asepsis was observed throughout the experiment. Single pieces of the tumor were loaded into hollow needles and ten normal white mice (young adults) were inoculated in the right groin. In loading the needles care was taken to macerate the tissue as little as possible. The control half of the tumor which received no x-rays was then inoculated into ten mice in exactly the same manner. In Experiment 1 the x-rayed half remained out of the body 45 to 50 minutes and the control half 1 hour. In Experiment 2 the x-rayed half remained out of the body 60 to 70 minutes and the control half $1\frac{1}{4}$ hours. The tumors resulting from the inoculations were measured at periods of 1 week



TEXT-FIG. 1. The generations of tumor transplantations for Series 1, extending over a period of 17 months. The final tumor transplant had been exposed to eleven treatments of x-rays during this period.

until the death of the mice. A healthy tumor was selected from the x-rayed series unless because of an epidemic or some other cause this was impossible, when one of the control tumors was chosen for treatment and an inoculation was made into another series of twenty mice in the manner described.

The first series of transplants was begun December 11, 1916, and terminated May 9, 1918 (Text-fig. 1). In Generations 5 and 6 the

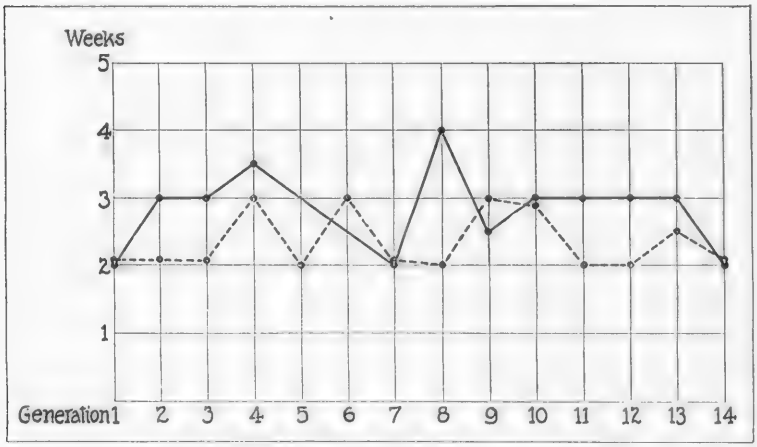


TEXT-FIG. 2. A comparison of the number of takes between x-rayed and untreated cancer on inoculation into mice. .....Controls. ————X-rayed cancers.

percentage of takes is inaccurate, probably much too low, on account of an epidemic among the mice which occurred before the usual time of appearance of tumors. For the transplants at this period, on account of the loss from the epidemic, the tumors of the next generation were taken from the control series. If we disregard the sudden drop in those two generations, the percentage of takes among the mice receiving x-rayed portions of the tumor remained between 25 and 45 during the first year, or nine generations. At the same time the percentage of takes among the control mice was between 50 and 70.

From the tenth generation to the fourteenth the percentage of takes does not remain consistently higher among controls and in both x-rayed and normal groups the percentage rises even to 100 (Text-fig. 2).

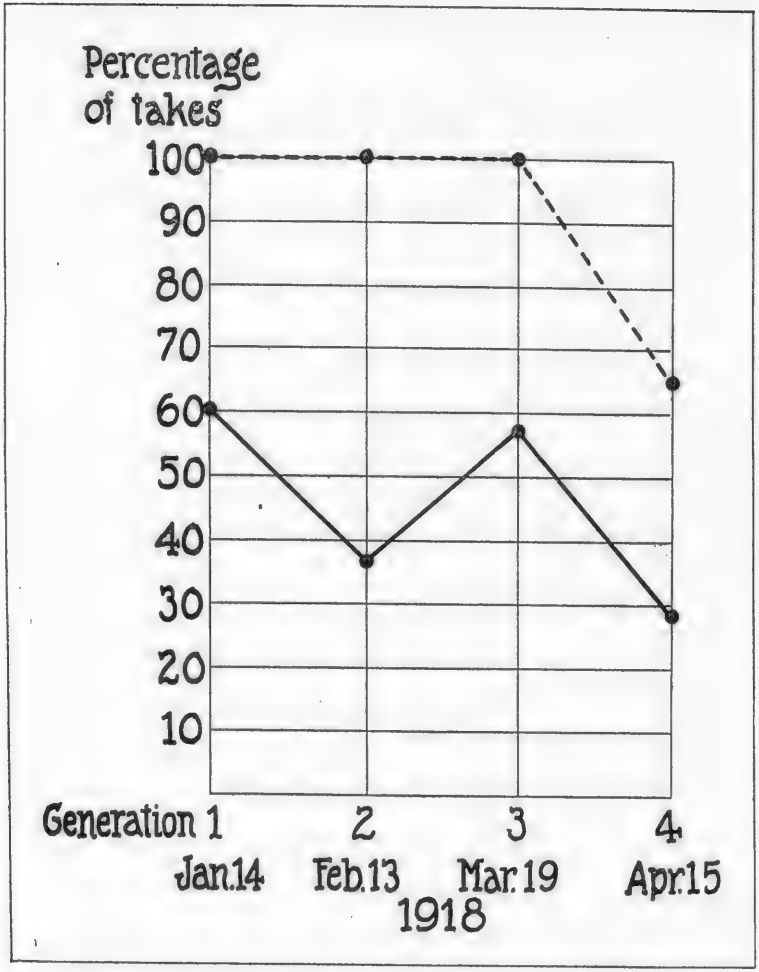
The rate of growth of the tumors was judged from curves representing the average time of appearance of the tumors and the average size 5 weeks after transplanting. As these two curves were essentially similar, only the former is illustrated (Text-fig. 3). In less than half the generation groups the tumors appeared a week earlier in the control mice than in those which had received the x-rayed



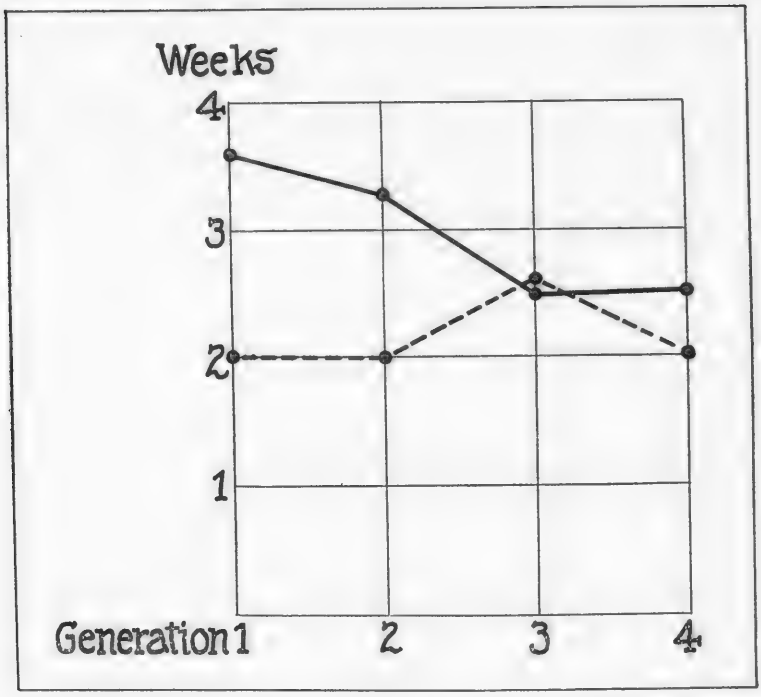
TEXT-FIG. 3. A comparison of the average time of appearance of tumors with and without exposure to x-rays. .....Controls. ————X-rayed cancers.

tumor. The difference in size between the tumors of the two groups 5 weeks after transplantation is slight and not consistent.

Experiment 2 was begun January 14, 1918, and terminated May 9, 1918. Four successive generations were observed. Without a much longer series of transplants it is impossible to judge the cumulative effect of this dose of x-rays on the general virulence of the tumor. The difference in the percentage of takes is consistently much more marked in this series than at any point in the first series (Text-fig. 4). Initially the rate of growth of the tumors receiving x-rays was also greatly retarded (Text-fig. 5).



TEXT-FIG. 4. A comparison of the percentage of takes for Series 2. .....Controls. ————X-rayed cancers.



TEXT-FIG. 5. Average time of appearance of tumors, x-rayed and untreated, in Series 2. .....Controls. ————X-rayed cancers.

DISCUSSION.

The present tendency of workers on x-ray therapy of cancer is to devise methods of increasing the amount of x-rays delivered at the location of the cancer process. In the light of our observations there is one point which should be taken into consideration; that is, whether or not we are justified in using a procedure which apparently only inhibits the cancer temporarily, while it incidentally lowers the resistance of the individual to the growth. It is well recognized that a proportion of cancers held in check for a time by x-ray treatment will later grow more rapidly. It is not possible to form an idea of what proportion of the total number of patients treated show this result, as few completely and accurately controlled series have been

published. Blood counts on a number of these individuals have been made in this laboratory, and they all showed remarkably low lymphoid counts. Our work, however, has been done mainly on cancer of mice, and we are therefore not warranted in drawing sweeping conclusions until there is more careful confirmation from studies on man. We feel justified, however, in suggesting that powerful doses of x-rays which are only capable of inhibiting cancer growth for a time may bring about eventually a lowered resistance to a return of the disease process.

CONCLUSIONS.

These experiments indicate that the direct action of x-rays in more powerful doses than can be applied therapeutically is somewhat injurious to tumor cells, but by no means destroys them. Experiment 1 also indicates that the cancer cells establish a resistance to the x-rays after repeated doses. This harmonizes with the experience of clinicians who have succeeded in checking cancerous growths for some time but reach a point where no response can be effected by repeated doses. The rays of low penetration used in Experiment 2 are apparently more harmful to tumor cells than the penetrating rays used in Experiment 1.

BLOOD COUNTS IN EXPERIMENTAL POLIOMYELITIS IN THE MONKEY.

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(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, October 30, 1918.)

When poliomyelitic virus has once been established in the monkey, it becomes more virulent for this animal by repeated passage.[1] Hence the virus used in experimental work usually brings about a severe form of the disease and high death rate. Occasionally an animal survives the stage of complete prostration if carefully attended and recovers with residual contractures. Experimental poliomyelitis in the monkey, then, is comparable with the severer forms in man, and observations on the variation in leucocyte count in the circulating blood would therefore be expected to yield more nearly uniform results than studies on human cases. In the latter there are many factors of difference in reaction and resistance, age and virulence of the virus, which might contribute to variation. Moreover, opportunities for observations during the incubation period in human cases are rare.

It is generally accepted that abnormal white blood counts are constantly found in poliomyelitis, but opinion is divided on the characteristics of this change.

Müller[2] asserts that a distinct leucopenia with a relative lymphocytosis is characteristic and pathognomonic of the febrile stage, while La Fétra[3] had previously described a moderate leucocytosis as being characteristic of the acute stage in human beings.

Gay and Lucas[4] summarize their blood findings as follows: "The acute stage of anterior poliomyelitis, as it occurs in human beings, and as it is produced experimentally in monkeys, is characterized by the occurrence of a distinct leuko-

[1] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.

[2] Müller, E., Die spinale Kinderlähmung, Berlin, 1910.

[3] La Fétra, L. E., *Arch. Pediat.*, 1909, xxvi, 328.

[4] Gay, F. P., and Lucas, W. P., *Arch. Int. Med.*, 1910, vi, 330.

penia. The differential count shows a relative increase in number of eosinophils and lymphocytes."

Peabody, Draper, and Dochez[5] found in human cases a constant and marked leucocytosis. They also found a constant increase in polymorphonuclear cells of 10 to 15 per cent and a diminution of lymphocytes of 15 to 20 per cent.

OBSERVATIONS.

Blood counts were made on six series of monkeys as follows: Series I, 40 normal monkeys for comparison (Table I); Series II, 4 monkeys already prostrate from experimental poliomyelitis (Table II); Series III, 12 monkeys during the incubation period, later developing the disease; Series IV, 5 monkeys which received the virus but did not develop the disease; Series V, 4 monkeys during the period of recovery from the acute stage; Series VI, 5 monkeys which had passed through the attack and recovered with residual paralyses.

In order to reduce the hourly variations in the blood counts the samples were collected at about the same time on the days of observation between 10.30 and 11.30 a.m.

Series I.—121 counts were made on 40 healthy, adult monkeys (*Macacus rhesus*). Averages of all counts are recorded in Table I.

TABLE I.

Average of 121 White and Differential Blood Counts on 40 Normal Monkeys.

White cells per c.mm.	Lymphocytes.		Polymorphonuclear leucocytes.			Large mononuclear and transitional leucocytes.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes per c.mm.
	Small.	Large.	Neutrophilic.	Eosinophilic.	Basophilic.				
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
22,181	47.5	6.2	41.1	4.1	0.4	0.6	53.7	11,815	9,116

Series II.—Seven counts on four monkeys paralyzed as a result of experimental inoculation with the virus of poliomyelitis are recorded in Table II.

[5] Peabody, F. W., Draper, G., and Dochez, A. R., A clinical study of acute poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, New York, 1912, 97.

TABLE II.

White and Differential Blood Counts on Monkeys Prostrate after Poliomyelitic Infection.

Monkey No.	Length of time after inoculation.	Day of prostration.	White cells per c.mm.	Lymphocytes.		Polymorphonuclear leucocytes.			Large mononuclear and transitional leucocytes.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes per c.mm.
				Small.	Large.	Neutrophilic.	Eosinophilic.	Basophilic.				
	days			*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*		
1	17	2	8,875	13.7	6.3	74.7	2.0	0.0	3.3	20.0	1,775	6,630
2	37	*	9,150	20.0	3.3	76.0	0.3	0.0	0.3	23.3	2,132	6,954
	38	1	8,275	24.3	2.7	70.0	3.0	0.0	0.0	27.0	2,234	5,793
	41	4	4,900	21.6	3.3	73.7	0.7	0.0	0.7	25.0	1,225	3,611
	47	10	4,675	28.3	18.7	53.0	0.0	0.0	0.0	47.0	2,197	2,478
3	10	2	17,550	15.7	1.7	82.7	0.0	0.0	0.0	17.4	3,054	14,514
4	10	3	21,600	14.0	5.7	80.3	0.0	0.0	0.0	19.7	4,255	17,345

* Almost prostrate; both legs flaccid; left arm and back weak.

Monkey 1 of this series received the virus by the nasal route. An intraspinal injection of 2 cc. of normal horse serum had been given on the day preceding the application of the virus to the nasal mucous membrane. After 10 days the monkey became excitable, after 11 days ataxic, and on the 15th day it became prostrate. The blood count was made on the 17th day after infection, which was the 8th day of the disease and the 2nd day after the monkey had become completely prostrated. Death occurred on the 21st day, and autopsy showed well defined lesions of poliomyelitis.

Monkey 2 was inoculated intracerebrally with an incubated mixture of 0.2 cc. of a Berkefeld filtrate of an active glycerolated virus and 2 cc. of human immune serum. The incubation period was unusually long, *viz.* 30 days; the course of the disease was also unusually slow, since the animal did not become prostrate until the 38th day after injection. When the first count was made on the 37th day after injection both legs were flaccid, and the left arm was weak. On the following day, when the animal became prostrate, another count was made. Other counts were made on the 41st and 47th days after injection, which were the 11th and 17th days of the disease. The animal succumbed on the 49th day, and microscopic lesions of poliomyelitis were found in the brain and cord.

Monkeys 3 and 4 were inoculated intracerebrally with an incubated mixture of 0.2 cc. of a Berkefeld filtrate of a 5 per cent suspension of an active glycerolated virus and 2 cc. of streptococcus immune rabbit serum 10 days before the blood

counts were made. Monkey 3 had been prostrate 2 days and Monkey 4 3 days at this time. Both animals were etherized on the 14th day, and autopsy showed characteristic lesions of poliomyelitis.

Series III.—Blood counts were made on twelve monkeys before inoculation with active virus, during the incubation period, and during the acute stage of poliomyelitis. Observations were made at intervals until the animals were killed by etherization or died of respiratory paralysis. All injections of the virus were intracerebral except in Monkeys 8 and 9, which received the virus by the nasal route on the day following an intraspinal injection of 2 cc. of normal horse serum. Nos. 5, 6, and 7 were from the Philippines, monkeys apparently closely similar to *Macacus cynomolgus*. The remainder were *Macacus rhesus*. The results are recorded in Table III. The variations in the circulating lymphocytes in Monkeys 5, 6, 7, and 8 are graphically represented in Text-fig. 1, *a*, *b*, *c*, and *d*, those of Monkeys 9 to 16 in Text-figs. 2 to 4.

Series IV.—Counts on five monkeys (Nos. 17 to 21), which received active virus by several routes but which did not develop the disease, are tabulated in Table IV. Fresh virus was given to Monkey 17 by mouth, to Monkey 18 by means of a nasal plug, to Monkey 19 by intrasciatic injection, and to Monkeys 20 and 21 by intracerebral inoculation.

Series V.—A summary of counts at intervals on monkeys during the acute stage and when partial or complete recovery had taken place is given in Table V. The fluctuations of the circulating lymphocytes are graphically represented in Text-figs. 5 and 6.

Monkey 22 of this series was injected intracerebrally with cultivated virus, first generation,[6] 4 days before the first blood count was made. At this time convulsions and ataxia were noted. On the 7th day after inoculation, when the third white blood count was made, the animal was partially paralyzed, but never became prostrate. 15 days after symptoms were first noted, at the time of the last blood count, the animal had almost completely recovered.

Monkey 23 was exposed to x-rays during the interval between the first and second blood counts, and the blood exhibited the characteristic lymphocytic drop.[7] Seven doses of unfiltered x-rays of 6 Holzknecht units each were given over

[6] Smillie, W. G., *J. Exp. Med.*, 1918, xxvii, 319.

[7] Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

TABLE III.

Blood Counts on Monkeys during the Incubation Period after Injections of Poliomyelitic Virus.

Monkey No.	Day of observation.	Length of time after inoculation.	Day of symptoms.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes.	
		days		*per cent*		*per cent*	
5*	1			24.0	6,780	74.7	21,103
	3	2		33.4	6,430	66.0	12,705
	4	3		47.7	7,966	51.7	8,643
	6	5	1	12.7	2,937	86.7	20,049
	7	6	2	44.7	9,834	54.3	11,946
	8	7	3	38.0	6,983	61.0	11,204
	9	8	4	20.7	5,988	79.3	22,938
	11	10	6	15.3	4,777	85.3	26,635
	14	13	9	12.3	3,795	85.3	26,465
6*	1			22.0	6,067	77.3	21,223
	3	2		27.0	3,692	72.3	9,887
	4	3		11.7	2,533	88.3	19,117
7*	1	3		34.7	10,315	61.3	18,221
	2	4		41.0	4,797	47.7	5,581
	4	6	1	10.0	1,948	90.0	17,528
8†	1			49.7	8,225	47.3	7,828
	2			51.0	6,464	45.3	5,742
	5			38.0	6,441	58.3	9,882
	9	4		60.7	8,270	38.3	5,218
	11	6		15.7	4,887	84.3	26,238
	13	8	1	42.3	7,254	56.0	9,604
	14	9	2	30.0	5,670	67.3	12,720
9†	1			47.7	9,922	49.7	10,347
	12	2	11	32.0	5,040	66.0	9,735
	13	3	12	38.4	5,126	60.7	13,230
	14	4	13	27.4	4,226	72.0	11,106
	15	5	14	40.7	4,701	58.0	6,699
	16	6	15	30.0	3,833	68.7	8,776
	18	8	17	30.0	4,583	70.0	10,693
	19	9	18	16.0	2,524	83.7	13,204

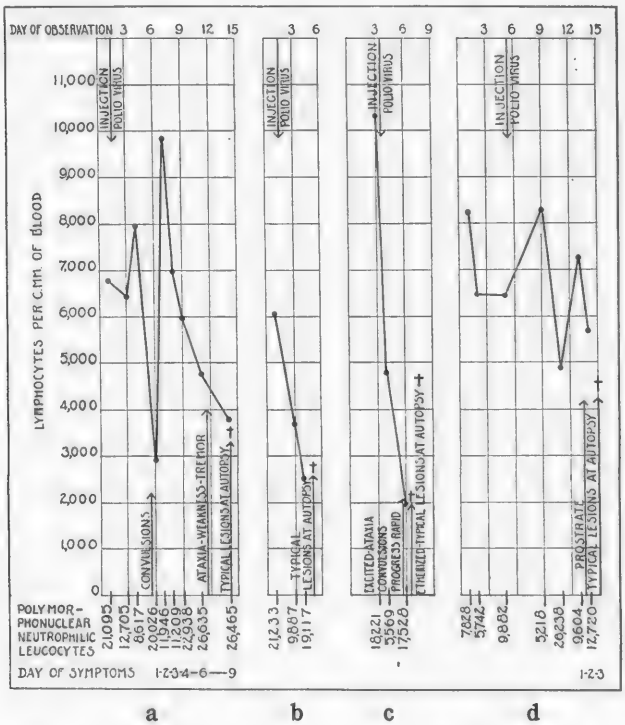
* Philippine monkey.

† Nasal route of infection. Previous intraspinal injection of 2 cc. of normal horse serum.

TABLE III—*Concluded.*

Monkey No.	Day of observation.	Length of time after inoculation.	Day of symptoms.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes.	
		days		*per cent*		*per cent*	
10	1	4		32.0	10,696	54.7	18,214
	3	6		51.5	15,939	47.1	14,578
	5	8	1	36.7	4,936	62.0	8,339
	6	9	2	23.0	3,835	73.7	12,290
	7	10	3	32.0	4,304	64.7	8,702
	8	11	4	29.0	3,567	68.7	8,450
	9	12	5	62.0	6,417	37.3	3,861
	10	13	6	33.7	4,457	66.3	8,768
11	1	2		46.7	12,117	49.3	12,793
	2	3		47.7	9,051	46.7	8,861
	5	6		44.0	8,217	53.7	10,029
	6	7	1	13.0	3,452	86.7	23,019
12	1			57.7	15,348	34.0	9,044
	3	2		60.0	10,740	37.0	6,623
	4	3		78.0	11,661	19.0	2,841
	5	4		69.3	19,477	28.3	3,870
	7	6		64.4	13,347	35.3	7,316
	8	7	1	19.7	6,161	79.7	24,926
13	1			37.3	12,476	62.3	20,839
	3	2		48.7	10,373	49.3	10,501
	6	5		70.7	9,297	28.7	3,744
	7	6		29.7	14,323	69.3	23,420
	8	7		36.7	5,633	63.0	9,671
	9	8	1	18.7	3,675	81.0	15,917
14				38.7	9,298	57.7	13,862
	8	7		22.4	3,489	76.7	11,946
	11	10	2	10.0	2,373	89.3	21,186
	12	11	3	20.0	2,970	80.0	11,880
	13	12	4	14.3	2,431	82.3	13,991
15	1	3		58.7	12,151	40.3	8,342
	4	6		46.7	17,046	51.7	18,871
	7	9	2	24.7	3,557	72.3	10,411
	8	10	3	17.7	3,615	79.3	16,197
16	1	4		45.0	8,179	53.3	9,687
	5	8	1	22.0	2,794	76.7	9,741
	6	9	2	14.7	2,132	83.7	12,137
	7	10	3	13.0	1,940	86.3	12,945
	8	11	4	11.0	2,540	87.0	20,097

a period of 6 days, the dorsal and ventral surfaces of the body being alternately exposed. Each dose was governed by the following factors: spark-gap 3 inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time of exposure 4 minutes. After these treatments the animal was inoculated intracerebrally with active virus and became completely prostrate

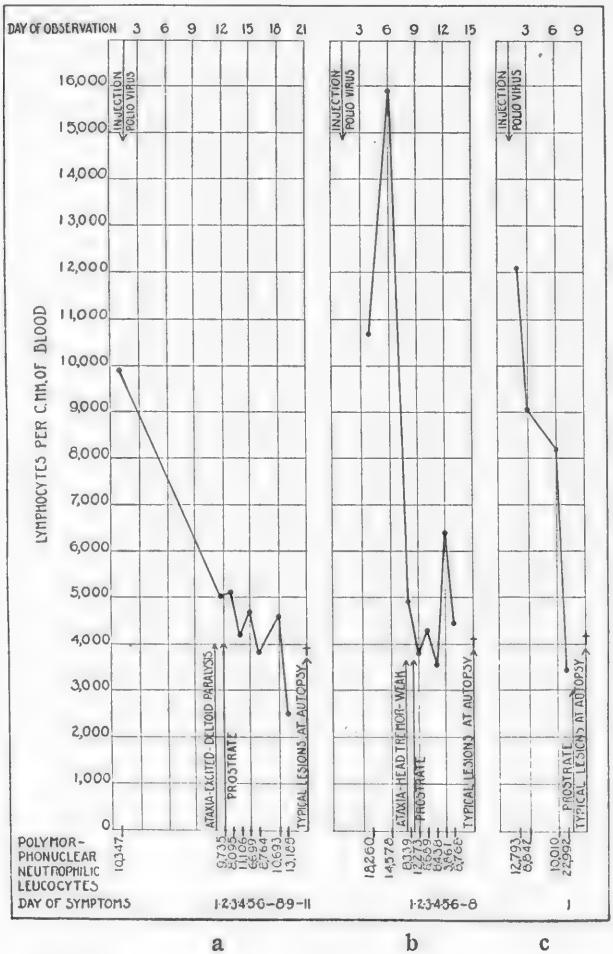


TEXT-FIG. 1, *a*, *b*, *c*, and *d*. Lymphocyte curve of Monkeys 5, 6, 7, and 8 (Table III).

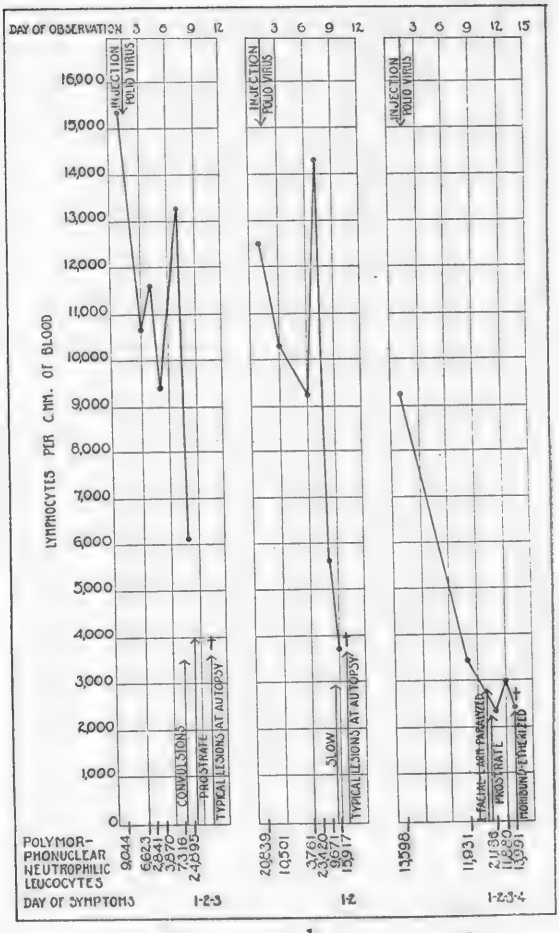
8 days later. The animal recovered with marked residual paralysis of both legs. Counts were made on the 59th day of observation, 45 days after prostration, and on the 76th day of observation.

Counts were made on Monkey 24 before injection and 5 days afterward. The animal was prostrate on the 12th day. Counts were made on the 16th, 28th, and 172nd days.

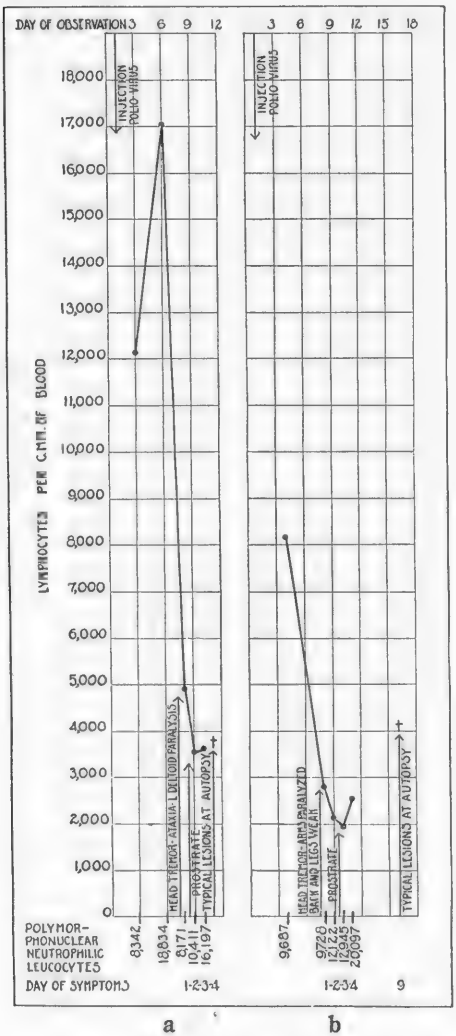
The first count was made on Monkey 25, 16 days after prostration, and the subsequent lymphocytic curve was followed for 22 days, during recovery with residual paralysis.



TEXT-FIG. 2, *a*, *b*, and *c*. (*a*) Lymphocyte curve of Monkey 9 (Table III). (*b*) Lymphocyte curve of Monkey 10 (Table III). (*c*) Lymphocyte curve of Monkey 11 (Table III).



TEXT-FIG. 3, *a*, *b*, and *c*. (*a*) Lymphocyte curve of Monkey 12 (Table III). (*b*) Lymphocyte curve of Monkey 13 (Table III). (*c*) Lymphocyte curve of Monkey 14 (Table III).



TEXT-FIG. 4, *a* and *b*. (*a*) Lymphocyte curve of Monkey 15 (Table III). (*b*) Lymphocyte curve of Monkey 16 (Table III).

TABLE IV.

Blood Counts on Monkeys Which Received Virus but Did Not Develop Symptoms.

Monkey No.	Day of observation.	Length of time after inoculation.	Day of symptoms.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes.	
		days		*per cent*		*per cent*	
17	1			41.0	9,615	57.0	12,367
	6	5		51.0	8,096	46.3	7,360
	13	12		31.4	4,561	64.7	9,398
	23	22		54.4	8,772	35.0	5,644
	37	36		46.7	8,556	45.0	8,556
18	1			50.7	11,205	40.3	8,906
	3	1		56.4	9,292	39.7	6,541
	6	4		49.3	8,849	47.0	8,437
	8	6		55.0	16,871	42.3	12,976
	11	9		68.3	16,871	28.3	6,055
	18	16		56.7	17,393	39.7	12,178
	27	25		55.4	11,426	43.0	8,869
	32	30		59.4	15,622	38.7	10,178
	35	33		47.7	9,922	49.7	10,347
19	1			43.3	9,959	51.7	11,891
	6	4		54.0	10,139	42.0	7,886
	8	6		42.7	7,163	54.7	9,178
	11	9		56.7	9,242	39.0	6,357
	13	11		50.0	7,188	43.3	6,224
	21	19		54.7	6,687	43.0	5,257
	31	29		43.0	7,740	56.3	10,134
	39	37		54.7	8,971	43.0	7,052
20	1			66.0	16,320	23.7	5,824
	3	2		71.3	11,889	26.3	4,386
	4	3		72.0	13,392	21.7	4,036
	5	4		66.0	9,719	27.7	4,079
	7	6		64.0	14,848	30.3	7,030
	8	7		62.0	10,804	31.7	5,524
	9	8		56.7	9,667	34.7	5,916
	11	10		58.0	8,715	36.7	5,514
	15	14		74.3	16,718	20.3	4,568
	18	17		59.3	10,674	36.0	6,480
	22	21		66.7	14,724	25.0	5,519
	26	25		74.3	13,764	18.0	3,334
	33	32		79.7	15,362	16.3	3,142
	36	35		72.3	16,792	23.3	5,178

TABLE IV—*Concluded.*

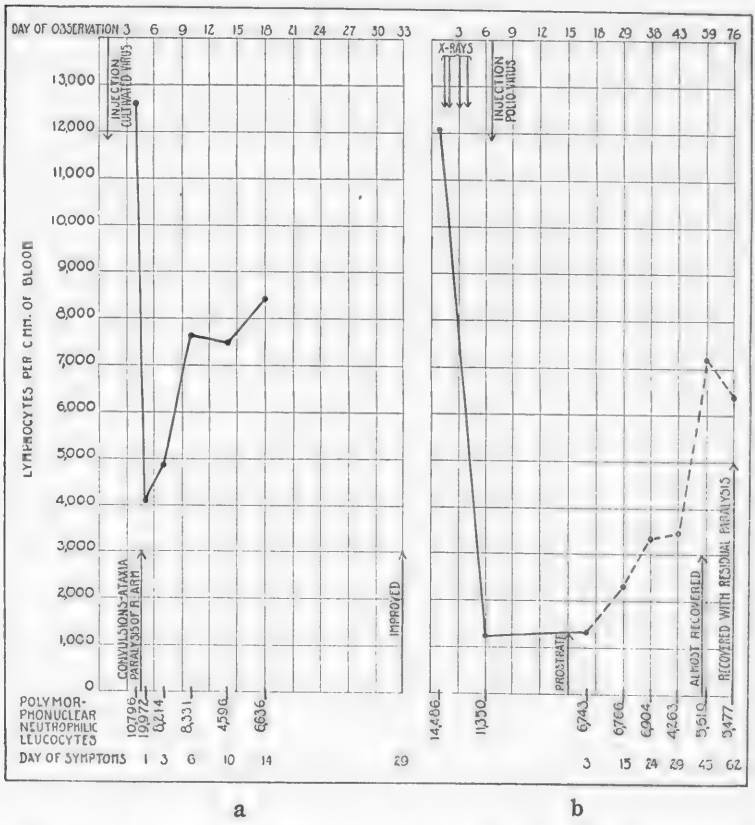
Monkey No.	Day of observation.	Length of time after inoculation.	Day of symptoms.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes.	
		days		*per cent*		*per cent*	
21	1			61.0	11,087	33.0	5,998
	2	1		30.7	4,313	66.3	9,315
	7	6		45.3	6,670	47.7	6,024
	8	7		62.3	7,507	35.0	4,218
	11	10		60.3	9,105	36.7	5,542
	14	13		67.3	11,592	30.7	5,288
	17	16		76.7	10,086	22.7	2,985
	21	20		64.0	10,304	34.0	5,474

TABLE V.

Blood Counts on Monkeys Observed during the Process of Recovery from Poliomyelitis.

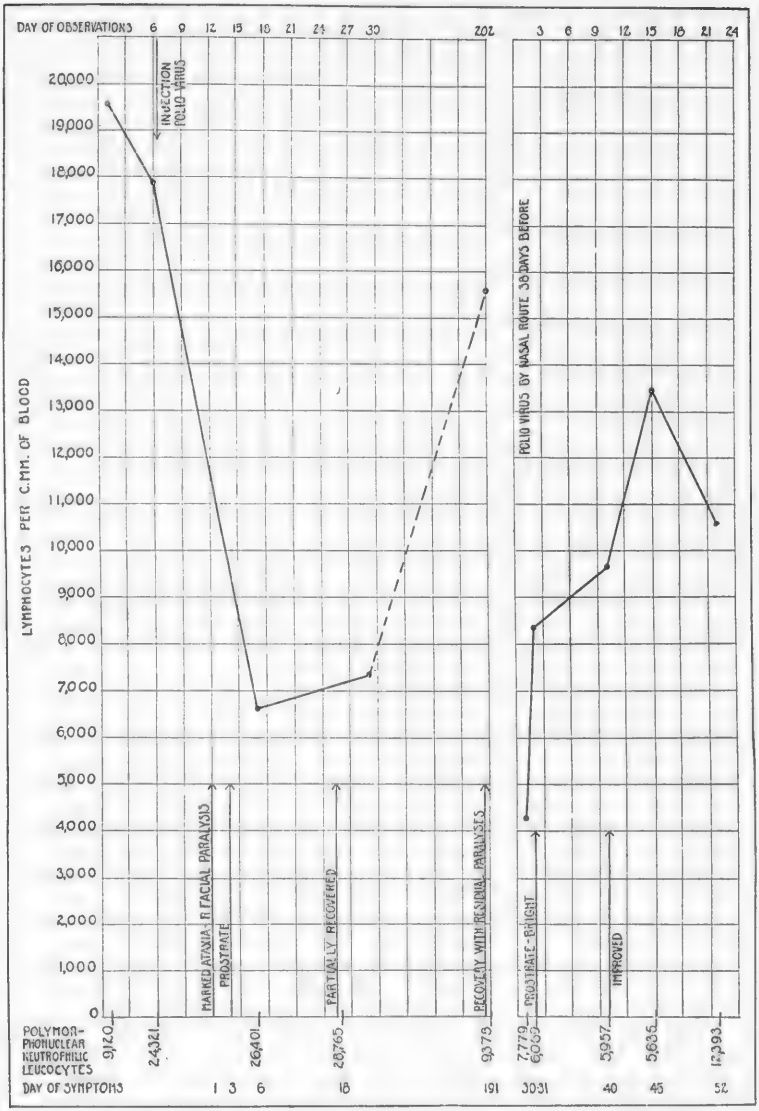
Monkey No.	Day of observation.	Length of time after inoculation.	Day of symptoms.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes.		Remarks.
		days		*per cent*		*per cent*		
22	1	4	1	55.3	12,619	45.7	10,820	Convulsions; ataxia.
	2	5	2	16.7	4,087	81.7	11,996	
	4	7	4	36.3	4,864	61.3	8,214	Paralyzed but not prostrate.
	7	10	7	29.3	7,640	70.3	18,331	
	11	14	11	62.3	7,476	38.3	4,596	
	15	18	15	55.0	8,429	43.3	6,636	Almost recovered.
23	1			45.0	12,071	54.0	14,486	X-rayed after count.
	6			9.0	1,231	83.0	11,350	
	17	11	3	16.0	1,332	81.0	6,743	Prostrate 3 days.
	29	23	15	23.3	2,318	68.0	6,766	Partly recovered.
	38	32	24	29.3	3,355	60.3	6,904	
	43	37	29	42.3	3,485	50.3	4,263	
	59	53	45	53.7	7,236	41.7	5,619	Almost recovered.
	76	70	62	50.3	6,363	43.3	5,477	Recovered with residual paralysis.
24	1			65.0	19,565	30.3	10,033	
	6	5		41.7	17,889	56.7	24,324	
	17	16	4	20.0	6,625	79.7	26,401	Prostrate 3 days.
	29	28	16	20.0	7,340	76.3	29,528	
	173	172	160	57.7	15,593	34.7	9,378	Recovered with residual paralysis.
25	1	30	16	29.3	3,289	69.3	7,779	Prostrate.
	2	31	17	53.7	7,357	44.3	6,069	
	10	39	25	56.7	8,661	39.0	5,957	
	15	44	30	67.0	12,462	30.3	5,636	
	22	51	37	41.7	9,570	54.0	12,393	Partial recovery with residual paralysis.

Series VI.—In Table VI are given blood counts made on monkeys which had completely recovered from acute poliomyelitis and proved immune to subsequent inoculations of active virus. All the animals



TEXT-FIG. 5, *a* and *b*. (*a*) Lymphocyte curve of Monkey 22 (Table V). (*b*) Lymphocyte curve of Monkey 23 (Table V).

had some residual contractures. Recovery, in the animals of this group, was of long duration, varying from 1 to 3 months at the time of the first blood count.



TEXT-FIG. 6, *a* and *b*. (*a*) Lymphocyte curve of Monkey 24 (Table V). (*b*) Lymphocyte curve of Monkey 25 (Table V).

TABLE VI.

Blood Counts on Monkeys Which Had Recovered from Acute Symptoms before the First Count.

Monkey No.	Day of observation.	Length of time since recovery.	Total lymphocytes.		Total polymorphonuclear neutrophilic leucocytes.	
		days	*per cent*		*per cent*	
26	1	72	52.7	8,550	43.7	7,090
	2	73	36.3	6,561	61.3	11,080
	5	76	55.0	6,463	40.3	4,735
27	1	72	57.7	7,746	28.0	3,759
	2	73	66.7	7,270	26.3	2,967
	5	76	72.0	7,200	20.0	2,000
28	1	92	50.7	4,700	45.7	4,277
	2	93	38.0	17,733	59.7	12,149
	5	96	43.3	6,712	54.0	8,370
29	1	84	46.3	7,281	47.3	7,438
	2	85	43.3	5,932	53.7	7,357
	6	89	40.3	8,181	52.3	10,617
	8	91	44.0	8,965	51.3	10,452
	9	92	52.7	7,879	42.7	6,384
	16	99	33.7	7,515	61.3	13,670
	20	103	42.3	8,238	50.3	9,796
	35	118	43.3	8,335	52.3	10,068
30	1	31	73.0	16,608	21.7	4,937
	2	32	67.0	10,318	29.0	4,466
	5	35	71.7	16,168	16.7	3,766
	16	46	39.0	14,182	60.3	20,381
	20	50	79.3	24,524	16.3	5,040

DISCUSSION.

All the blood counts made on monkeys during the course of typical acute experimental poliomyelitis show a variation from the normal. This change is apparent in Table VII,[8] in which the average lymphocytes and polymorphonuclear counts and percentage during the acute stage are compared with normal averages. There are included aver-

[8] Counts on Monkey 6 (Table III) are not included in the computation.

ages at the time of the highest and also of the lowest lymphocyte count. The variations are sufficiently great to warrant definite conclusions.

After injection of active poliomyelitic virus the lymphocytes are diminished but return to their former number and are actually increased between the 4th and 6th days of the incubation period (Table III). The polymorphonuclear count is high at this time. The normal average of lymphocytes of 11,815 (Table I) is increased to an average of 19,696 though the average percentage is slightly lowered. During the first 3 days after onset a marked diminution in the lymphocytes takes place. Thus, instead of an average normal lymphocytic count of 11,815, the number is 3,302, and the average percentage

TABLE VII.

Average Variation in White Cells during Infection with Poliomyelitis.

Status of monkeys at time of counts.	No. of animals studied.	Total No. of counts.	Large and small lymphocytes. Average.	Average total No. of lymphocytes per c.mm.	Average total No. of polymorphonuclear neutrophilic leucocytes.	
			per cent		*per cent*	
Normal	40	121	53.7	11,815	41.1	9,116
Incubation period at time of highest lymphocytic count	12	12	44.0	19,696	54.3	13,383
In acute stage at time of lowest lymphocytic count	12	12	15.6	3,302	83.7	18,231
Prostrate	4	6	21.9	2,758	76.5	10,800
Recovered with residual paralysis	6	22	53.2	9,026	41.3	6,800

is 15.6. At this time the polymorphonuclear neutrophilic leucocytes are materially increased in number (18,231) as compared with an average normal of 9,116, and the percentage is also increased from an average of 41 to 84 per cent of the total white cells. When the monkeys become completely prostrate metabolism is at low ebb, and there occurs a further decrease in the actual number of lymphocytes (Table III) which remains low for long periods (Tables II and V). The total number of polymorphonuclear neutrophilic leucocytes returns to normal, but there remains a relative increase averaging 77 per cent. Finally, during recovery both types of cells return to the average normal count and relation. No stimulation of the lympho-

cytes above normal appears during recovery. The counts made on one animal (Monkey 24, Table V) at 160 days after onset are almost the same as those made before the injection of virus.

In the monkeys receiving virus but not developing symptoms there seemed to be a constant tendency for the lymphocytes to decrease following the administration of the virus (Table IV). This decrease was followed by a gradual return to normal.

Monkey 23 (Table V) was x-rayed before inoculation with the virus of poliomyelitis, and the return of the circulating lymphocytes to their normal level, in this animal, seemed to be considerably delayed. As the x-rays have a depleting effect on the circulating lymphocytes[9] similar to that exhibited by the virus of poliomyelitis, the protracted recovery may, in this instance, depend on a summative action of the two agents.

Monkey 22 (Table V) was also included in the series used by Smillie[6] in attempts to produce poliomyelitis with cultivated virus. Typical pathological lesions were not observed at autopsy; however, the symptoms and the blood curve simulating those observed in known poliomyelitic monkeys are suggestive and offer possible additional proof that the monkey developed mild poliomyelitis after intracerebral inoculation with the fourth generation of a culture of the globoid bodies and recovered before it was etherized for autopsy.

SUMMARY AND CONCLUSIONS.

Blood counts were made on six series of monkeys before and at various intervals after the injection of active poliomyelitic virus. From the data thus obtained the variations in the circulating white cells have been followed in the several stages of the disease (*a*) before injection, (*b*) during the incubation period, (*c*) during the acute stage, including the stage of prostration, and (*d*) during recovery. On account of experimental conditions not all the monkeys were observed during the four periods, so that averages of counts in several monkeys are used for comparison.

Averages of 121 counts on 40 normal monkeys are recorded.

[9] Amoss, H. L., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, xxix, 115.

Immediately following the injection of the virus the relative and actual numbers of lymphocytes are slightly diminished. In many cases the curve continues sharply downward. In others from the 4th to the 6th day there is an actual increase for a brief period to a point somewhat in excess of the original count. With the onset of symptoms a lymphocytic crisis takes place. The curve then continues slightly downward, while the polymorphonuclear neutrophilic leucocytes are relatively and actually increased at approximately the same time. During the stage of prostration the curve of the polymorphonuclear neutrophilic leucocytes returns to almost normal, while the lymphocytic curve continues slightly downward. With recovery the lymphocytes slowly return to normal after several weeks. There is no evidence of lymphocytic stimulation after recovery.

Eosinophilic, basophilic, large mononuclear, and transitional leucocytes follow the depressions and stimulations exhibited by the neutrophilic cells of the same series.

The results here recorded are consistent with the observations of Peabody, Draper, and Dochez on human cases.

The increase in the total number of circulating lymphocytes after the lymphocytic crisis is coincident with the passing of the acute stage.

Additional evidence is presented to indicate that Smillie produced atypical but definite poliomyelitis in the monkey with cultivated virus.

EFFECTS OF LARGE DOSES OF X-RAYS ON THE SUSCEPTIBILITY OF THE MONKEY TO EXPERIMENTAL POLIOMYELITIS.

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The ready communicability of poliomyelitis among human beings is generally accepted save by a certain few who disregard the idea of selective susceptibility. It is a matter of common observation that of many persons of the most susceptible age definitely exposed only a small percentage contracts the disease. For example, in the New York City epidemic of 1916 there were approximately 1,200,000 individuals of the more susceptible ages, *i.e.* less than 16 years,[1] exposed, but only 8,750 of this age in a total of 9,023 cases were reported. In the epidemic in Westchester County single cases in families of several children were the rule, although in uncommon instances there were three or four cases in the same family. This contrasts strongly with the experience in measles, a disease of practically no selective action. The reasons for the differential power of poliomyelitis to attack are not apparent; however, three facts have been lately adduced which may have some bearing on the problem. In the first place, it has been shown[2] that the route of infection with the virus by the blood stream in monkeys, which is ordinarily closed, except when massive doses are used, can be traversed by smaller doses after intraspinal injection of substances setting up an aseptic meningitis. Infection by the nasal route is also rendered much easier by similar preparation. Flexner and Amoss[3] suggest that possibly one reason for infection or non-

[1] The epidemic of poliomyelitis (infantile paralysis) in New York City in 1916, Department of Health of the City of New York, Monograph series, No. 16, New York, 1917.

[2] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1914, xx, 249.

[3] Flexner and Amoss, *J. Exp. Med.*, 1917, xxv, 525.

infection in persons exposed may lie in the patency or continuity, as the case may be, of the meningeal-choroidal complex. Secondly, Zingher[4] has shown that whereas only 30 per cent of apparently normal children yield a positive reaction to intradermal injection of diphtheria toxin, about 80 per cent of persons recently recovered from poliomyelitis give a positive reaction. This suggests either a general state of lowered resistance, as indicated by susceptibility to both poliomyelitis and diphtheria, or less probably that infection with the former reduces resistance to the latter. Thirdly, Amoss and Taylor[5] noted the power of nasal washings from certain individuals to neutralize the virus of poliomyelitis and suggest that this action may be the first line of defense against poliomyelitic infection.

No definite data have been presented to indicate variations in resistance to poliomyelitic virus among monkeys, except perhaps the impression that tuberculous animals seem to be more susceptible. Moreover, the conditions of the experimental infection are purposely arranged to reduce the chance of variations in individual resistance. The virus when it has once been adapted to the monkey becomes highly infective and remains so during many animal transfers. Finally, however, after several years passage the virulence diminishes.[6] The original M. A. strain adapted to monkeys by Flexner and Lewis[7] in 1909 now possesses less infective power and lends itself to observations on susceptibility in experimental poliomyelitis.

The observations of Peabody, Draper, and Dochez[8] on human cases and of Taylor[9] in experimental poliomyelitis with regard to the remarkable lymphocytic changes accompanying the infection and the focal infiltrations of these cells as one of the histological characteristics of the disease suggest an intimate relation between the infection and the circulating lymphocytes. It may be possible by bring-

[4] Zingher, A., *Dept. Health, City of New York, Reprint Series, No. 52*, 1917.

[5] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, xxv, 507.

[6] Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, xix, 195.

[7] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 1639.

[8] Peabody, F. W., Draper, G., and Dochez, A. R., A clinical study of acute poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, New York, 1912.

[9] Taylor, H. D., *J. Exp. Med.*, 1919, xxix, 97.

ing about conditions in which the lymphocytes are greatly diminished to render the monkey more susceptible to poliomyelitic virus. The experiments here recorded support this belief.

EXPERIMENTAL.

By exposure to properly controlled doses of x-rays it is possible to diminish the circulating lymphocytes.[10] Accordingly, two normal monkeys were selected for each experiment. One was treated with x-rays[11] daily or every other day until the total number of lymphocytes per c.mm. of blood was between 1,000 and 2,000. A Berkefeld filtrate of a 5 per cent suspension of glycerolated M. A. virus was injected intracerebrally into the x-rayed and the control animal. The dose of virus was previously titrated and known to be subinfective. Should the dose of the virus be too large, both animals are infected, and if the maximum subinfective dose is reduced by more than one-half, the more susceptible x-rayed animal does not respond with an attack of poliomyelitis.

Three series of experiments were carried out according to the plan outlined. The protocols follow.

Series I.—Macacus rhesus A (control). Jan. 17, 1917. Blood count, 7,750 lymphocytes and 3,950 polymorphonuclear neutrophilic leucocytes per c.mm. Injected intracerebrally with 1 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord (M. A. virus). The monkey remained well.

Macacus rhesus B. Jan. 3, 1917. Blood count, 6,930 lymphocytes and 8,465 polymorphonuclear neutrophilic leucocytes per c.mm. Received seven doses of x-rays, 6 Holzknecht units each. Jan. 15. Blood count, 1,257 lymphocytes and 4,300 polymorphonuclear neutrophilic leucocytes per c.mm. (Text-fig. 1, *a*). Jan. 17. Received intracerebrally (at the same time as the control injection) 1 cc. of the Berkefeld filtrate of M. A. virus used in Monkey A. Jan. 23. Excitable; left arm paralyzed; right deltoid weak. Jan. 24. Prostrate. Jan. 27. Moribund; etherized.

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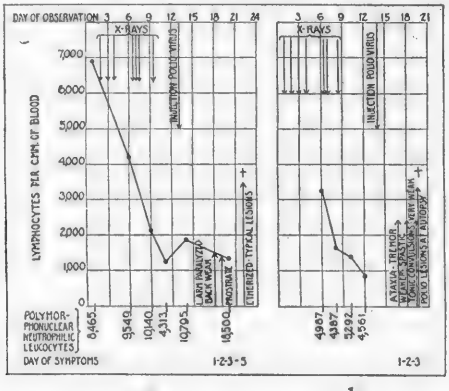
[10] Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, xxix, 53.

[11] Unfiltered x-rays of 6 Holzknecht units each were given at each dose alternately on the dorsal and ventral surfaces. Each dose was governed by the following factors: spark-gap 3 inches, milliamperes 10, distance from the target of the Coolidge tube to the skin 12 inches, and time of exposure 4 minutes.

Autopsy.—Severe acute lesions of poliomyelitis, perivascular infiltration, and necrosis of ganglion cells.

Series II.—Macacus rhesus C (control). Feb. 5, 1917. Blood count showed 16,630 lymphocytes and 4,050 polymorphonuclear neutrophilic leucocytes per c.mm. Injected intracerebrally with 1 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord (M. A. virus). The monkey remained well. Mar. 5. Began daily treatments with x-rays. After seven doses the lymphocytes were reduced in number to 878 per c.mm., but no symptoms of poliomyelitis developed. 6 months later the animal showed symptoms of tuberculosis and died.

Autopsy.—Marked tuberculosis of lungs and abdominal viscera.



a b

TEXT-FIG. 1, *a* and *b*. (*a*) Lymphocytic curve of Monkey B, Series I. (*b*) Lymphocytic curve of Monkey D, Series II.

Macacus rhesus D. Blood was not counted before treatment with x-rays. On Feb. 5, 1917, seven doses of x-rays had been given, and the blood count was as follows: lymphocytes 885 (15 per cent), polymorphonuclear neutrophilic leucocytes 4,561 (77.3 per cent) (Text-fig. 1, *b*). On this date there was injected intracerebrally 0.75 cc. of the Berkefeld filtrate of M. A. virus given Monkey C (control). Feb. 8. Ataxic; protects right leg; marked head tremor. Feb. 9. Weaker; ataxia increased. Feb. 10, a.m. Back and both legs weak; convulsion lasting 2 minutes. 2 p.m. Found dead.

Autopsy.—All the organs very pale; spleen very small. Brain and cord showed intense congestion of gray matter. Microscopic examination showed focal infiltrations and perivascular infiltration of cells, most of which were polymorphonuclear leucocytes. The lesions in the cord and spinal ganglia were not marked.

Macacus rhesus E. Blood count before exposure to x-rays, 8,785 (43.6 per cent) lymphocytes and 8,180 (40.6 per cent) polymorphonuclear neutrophilic leucocytes. Received eight doses of x-rays. Feb. 5, 1917. The blood count was as follows: 581 (9 per cent) lymphocytes and 4,810 (74.6 per cent) polymorphonuclear leucocytes. Injected intracerebrally with 0.5 cc. of the poliomyelitic virus filtrate. The animal never showed symptoms of poliomyelitis.

In this experiment the control showed no symptoms after receiving 1 cc. of the virus filtrate, while the animal treated with x-rays, receiving only 0.75 cc., succumbed to poliomyelitis. The control animal after 28 days was treated with x-rays, but no symptoms developed, indicating the destruction or removal of the virus in that time.

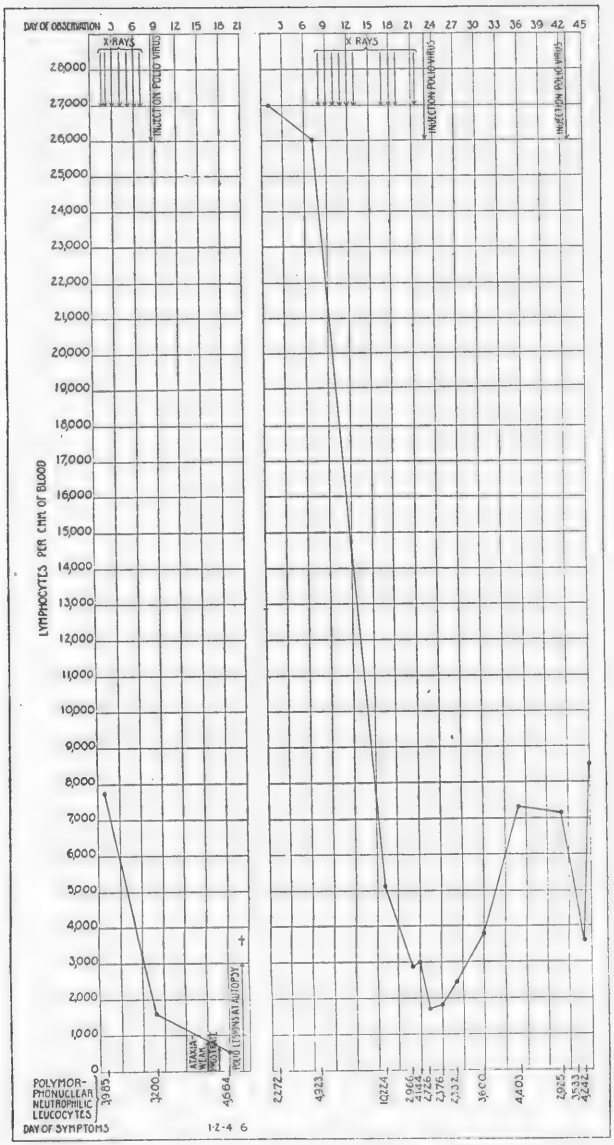
Series III.—Macacus rhesus F (control). Apr. 3, 1918. Blood count, lymphocytes 8,432 (42 per cent) and polymorphonuclear neutrophilic leucocytes 11,162 (55.6 per cent) per c.mm. Injected intracerebrally with 0.5 cc. of a Berkefeld filtrate of a 5 per cent suspension of glycerolated poliomyelitic monkey cord (M. A. virus). The animal showed no symptoms of poliomyelitis, but later died of tuberculosis.

Autopsy.—Almost total involvement of left lung and tuberculosis of abdominal viscera. There was no evidence of poliomyelitic lesion in the brain or cord.

Macacus rhesus G. Mar. 26, 1918. Blood count, 7,751 (59.7 per cent) lymphocytes and 3,985 (30.3 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. Received daily treatments with x-rays, 6 Holzknecht units each, for 7 days. Apr. 3. Blood count, 1,625 (14.7 per cent) lymphocytes and 3,200 (75.3 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. (Text-fig. 2, *a*). Injected intracerebrally with 0.5 cc. of the Berkefeld suspension of the glycerolated poliomyelitic cord (M. A. virus) given Monkey F. Apr. 10. Ataxic; head tremor; partial paralysis of left arm, right deltoid, and back. Apr. 11. Completely prostrate. Apr. 13. Blood count, 520 (10 per cent) lymphocytes and 4,664 (89.7 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. Apr. 15. Found dead.

Autopsy.—Slight perivascular infiltration and nerve cell degeneration in the medulla and slight focal accumulations of cells, most of which were polymorphonuclear neutrophilic leucocytes.

The outcome of this experiment supports the findings in Series I, in which the control animal remained unaffected by a dose of M. A. poliomyelitic virus which caused complete paralysis in a monkey which had been treated with x-rays with a consequent decrease in the circulating lymphocytes.



TEXT-FIG. 2, *a* and *b*. (*a*) Lymphocytic curve of Monkey G, Series III. (*b*) Lymphocytic curve of Monkey I.

Survival of a Subinfective Dose of the Virus in Normal Monkey Brain.

The usual incubation period in experimental poliomyelitis is from 7 to 10 days; however, when a weak virus[6] is used, or in neutralization experiments[5] this period may be prolonged to 30 or 40 days. An incubation period longer than 30 days is unusual. It is presumed that the virus lay dormant for the prolonged period or multiplied slowly. Multiple injections of virus weakened by long storage in 50 per cent glycerol[12] apparently lessen rather than increase the resistance of the monkey to subsequent injections, if the interval between injections is about 7 days. Longer periods have not been studied. In monkey poliomyelitic brain removed from the body and placed under anaerobic conditions at 37°C. in tissue ascitic fluid the virus may survive without multiplication for 20, but not for 30 days.[13] The virus does not survive 7 days *in vivo* in rabbit brain[14] or even for 2 days in the rat brain.[15] Is the subinfective dose then disposed of in a monkey of normal resistance within the maximum incubation period? The following experiments have a bearing on this question.

Experiment 1.—Monkey C, used as control in Series II, received 1 cc. of virus filtrate intracerebrally. When 28 days had elapsed the animal was exposed to x-rays until the lymphocytes had decreased tq 878 per c.mm. The animal had no symptoms of poliomyelitis.

Experiment 2.—Macacus rhesus H. Mar. 23, 1917. Received 1 cc. of filtrate of suspension of virus used in Experiment 1 and at the end of 15 days was exposed to x-rays, which reduced the lymphocytes to 1,125 per c.mm. No symptoms of poliomyelitis developed in the animal.

Apparently in the animals of normal resistance subinfective doses are disposed of in 15 days or are so weakened that reduction in resistance of the animal does not permit of infection. The experiments were not carried further because of lack of monkeys due to war conditions.

[12] Flexner and Amoss, *J. Exp. Med.*, 1917, xxv, 539.
[13] Flexner and Amoss, *J. Exp. Med.*, 1915, xxi, 509.
[14] Amoss, H. L., *J. Exp. Med.*, 1918, xxvii, 443.
[15] Amoss, H. L., and Haselbauer, P., *J. Exp. Med.*, 1918, xxviii, 429.

Is Immunity Once Established Reduced by Exposure to X-Rays?

Flexner and Lewis[16] have shown that monkeys which have passed through an attack of poliomyelitis are immune to subsequent injections of an active virus. Moreover, sera from immune monkeys and from human beings[17] who have had the disease possess definite neutralizing power for the poliomyelitic virus. It is probable that the immunity could not be destroyed by any of the factors accompanying exposure to x-rays. A single experiment was carried out on a monkey paralyzed by previous injections of poliomyelitic virus.

Experiment 3.—Macacus rhesus I (active immune) had received dried poliomyelitic virus on Feb. 10, 1917, showed symptoms on Feb. 21, and had complete paralysis of both legs and deltoid muscles on Feb. 23. The animal gradually improved with residual paralysis of both legs. Apr. 6. Blood count, 26,062 (81 per cent) lymphocytes and 4,920 (15.3 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. X-ray treatments were begun. During the succeeding 14 days ten exposures, of 6 Holzknecht units each, were made. Apr. 24. The blood count was as follows: 1,739 (37 per cent) lymphocytes and 2,726 (58 per cent) polymorphonuclear neutrophilic leucocytes per c.mm. Apr. 25. Injected intracerebrally with 1 cc. of a 5 per cent suspension of fresh poliomyelitic monkey cord. No symptoms developed. May 15. Injected intracerebrally with 2 cc. of 5 per cent suspension of active poliomyelitic monkey cord. The animal remained as before without any increase in paralysis or other symptoms of poliomyelitis. The lymphocytic changes are graphically recorded in Text-fig. 2, *b*.

In this experiment exposure to x-rays sufficient to reduce the circulating lymphocytes to one-fifteenth of their original number failed to destroy immunity in the monkey established by a previous attack of poliomyelitis.

SUMMARY AND CONCLUSIONS.

In two series of the experiments here recorded the monkey which had been repeatedly exposed to x-rays responded with typical acute poliomyelitis to an intracerebral inoculation of poliomyelitic virus fil-

[16] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, lv, 662.

[17] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, lxviii, 311. Römer, P. H., and Joseph, K., *Münch. med. Woch.*, 1910, lvii, 568. Flexner and Lewis, *J. Am. Med. Assn.*, 1910, liv, 1780. Anderson, J. F., and Frost, W. H., *J. Am. Med. Assn.*, 1911, lvi, 663.

trate, whereas the normal control receiving the same dose showed no symptoms. In another series the x-rayed animal came down with typical poliomyelitis after inoculation with three-fourths of the dose which was not infective for the control. It has been demonstrated that the x-rays diminished both the number of circulating lymphocytes and the resistance of the animal to the weak poliomyelitic virus. Whether the lowered resistance of the animals as the result of the treatment with x-rays is due to the reduction of circulating lymphocytes in each of the x-rayed monkeys is not determined in these experiments.

However, the great reduction in lymphocytes in human cases[8] and in monkeys during the acute stage of experimental poliomyelitis[9] and the gradual return of the cells to their former numbers during recovery strongly suggest a definite relation between these cells and one factor of resistance in poliomyelitis.

On the other hand, the reduction in resistance by x-rays, while definite, is not sufficiently great to warrant the conclusion that we are dealing with major factors governing infection or non-infection.

Another experiment in this paper deals with the survival of a subinfective dose of the virus in the normal monkey brain. A monkey receiving the subinfective dose of the virus was exposed to x-rays at 28 days, another at 15 days after injection, but neither animal showed symptoms of poliomyelitis. It is concluded that within this period the virus did not remain unchanged in the normal monkey brain.

An attempt to reduce the immunity in a monkey acquired by an attack of experimental poliomyelitis was unsuccessful.

THE ACTION OF CHLORINATED ANTISEPTICS ON BLOOD CLOT.

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Experiments recently reported from this laboratory[1] demonstrated that hypochlorite solutions, in the concentration and alkalinity used clinically, exhibit a solvent action on necrotic tissue. Fiessinger and his coworkers[2] have reported a similar action of these solutions on pus cells. The importance of the latter observation is emphasized by the experiments of Rous and Jones,[3] which demonstrated that intact leucocytes may protect bacteria which they have phagocyted from the action of an antiseptic. These bacteria, when liberated after autolysis of the leucocytes, are viable and may proliferate in an apparently normal manner.

As the hypochlorite solutions are extensively used in the treatment of infected wounds, and as their cleansing properties have been emphasized, it seemed important to determine definitely whether these solutions were effective in dissolving or penetrating blood clot. The possibility that clotted blood in wounds might serve as a protective covering for virulent microorganisms, thereby preventing the bactericidal action of the antiseptic employed, seemed worthy of investigation.

As it is generally recognized, and as early experiments seemed to demonstrate conclusively, that solutions of a degree of concentration and alkalinity compatible with clinical use exhibited little solvent action on blood clot, certain modifications were later introduced in an endeavor to influence the solutions in this direction.

[1] Taylor, H. D., and Austin, J. H., *J. Exp. Med.*, 1918, xxvii, 155. Austin, J. H., and Taylor, H. D., *ibid.*, 627.

[2] Fiessinger, N., Moiroud, P., Guillaumin, C.-O., and Vienne, G., *Ann. méd.*, 1916, iii, 133.

[3] Rous, P., and Jones, F. S., *J. Exp. Med.*, 1916, xxiii, 601.

Method.—Rabbit blood was allowed to clot in test-tubes, and after separation from the serum the remaining cylindrical mass was cut into a number of discs, each 1 cm. in thickness and 1.5 cm. in diameter. Each disc was then placed in 50 cc. of the solution to be tested for varying lengths of time. The appearance and size of the discs were noted before and after exposure to the action of the solution and examined carefully for any evidence of dissolution or penetration by the antiseptics. Careful controls were included in every experiment.

EXPERIMENTAL.

Experiment 1.—The first series of experiments was of a morphological nature, and an effort was made to determine by the appearance of the blood clot discs whether or not any solvent action was demonstrable. Dakin's hypochlorite (0.5 per cent sodium hypochlorite), chloramine-T (2 per cent), and dichloramine-T (5 per cent in chlorcosane) solutions apparently had no solvent, disintegrative, or penetrative action on the discs when allowed to act for periods of time varying from 15 minutes to 12 hours.

Experiment 2.—In the hope of varying the permeability of the blood clots, they were first treated with $\frac{M}{8}$ calcium chloride and $\frac{M}{8}$ sodium chloride. Loeb[4] has already shown that calcium chloride increases and sodium chloride decreases the permeability of masses of finely powdered gelatin. After varying lengths of time in the salt solutions (from 15 minutes to 2 hours), the clots were transferred to the antiseptic solutions, hypochlorite 0.5 per cent, chloramine-T 2 per cent, dichloramine-T 5 per cent in chlorcosane, and in distilled water, and allowed to remain in contact with these solutions for from 15 minutes to 12 hours. No appreciable action was noticeable except all absence of hardening of the surface layer of the clot first treated with calcium chloride and later transferred to Dakin's hypochlorite solution. It was then decided to test the penetration of the solutions in a manner allowing objective analysis, and the following experiments were performed in the hope of getting accurate and comparable figures with regard to the relative solvent powers of certain antiseptics.

Experiment 3.—10 cc. of sterile rabbit blood were placed in a sterile test-tube containing 0.5 cc. of a 24 hour bouillon culture of *Staphylococcus aureus* and shaken thoroughly against a sterile rubber stopper to insure even distribution of the bacteria through the resulting clot. After clotting and separation of the serum the cylindrical mass was cut, with precautions against further contamination, into equal sized discs, 1 cm. in thickness and 1.5 cm. in diameter. The discs were then placed in bottles containing 50 cc. of the following solutions: (1) Dakin's hypochlorite solution 0.5 per cent; (2) chloramine-T solution 2 per cent; and (3)

[4] Loeb, J., *J. Biol. Chem.*, 1917, xxxi, 343.

sterile salt solution, as control. A disc was removed from each bottle after a half hour and a second after a 1 hour interval. The discs were then washed in two changes of sterile saline solution to remove all traces of the test solution; the second saline wash contained a few drops of a $\frac{N}{10}$ sodium thiosulfate solution to neutralize any chlorine remaining. Each disc was thoroughly ground in a sterile mortar with 5 cc. of saline solution, agar tubes were inoculated with two loopfuls of the resulting emulsion, and plates poured. After 24 hours incubation at 37°C. the colonies developing in the plates were counted, and the results are summarized in Table I.

TABLE I.

Time of contact.	Dakin's solution.	Chloramine-T solution.	Salt solution control.
hrs.			
$\frac{1}{2}$	60	100	Confluent.
1	60	100	"

Experiments 4 and 5.—These experiments were similar to Experiment 3, and the results are recorded in Tables II and III.

TABLE II.

Time of contact.	Dakin's solution.	Chloramine-T solution.	Salt solution control.
hrs.			
$\frac{1}{2}$	300	312	Confluent.
1	280	Confluent.	"
2	Confluent.	300	"

TABLE III.

Time of contact.	Dakin's solution.	Chloramine-T solution.	Salt solution control.
hrs.			
1	Confluent.	Confluent.	Confluent.
2	"	"	"

Experiment 6.—Discs prepared as in Experiments 3 to 5 were placed in $\frac{M}{8}$ calcium chloride and $\frac{M}{8}$ sodium chloride for from 1 to 2 hours and later transferred to Dakin's solution to test the effect of the saline solution on the permeability of the clot. The results are given in Table IV.

TABLE IV.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 calcium chloride.	1	Dakin's hypochlorite.	2	222
M/8 " "	1		0	Confluent.
Dakin's hypochlorite.	2		0	800
M/8 sodium chloride.	1	Dakin's hypochlorite.	2	545
M/8 " "	1		0	Confluent.

Experiment 7.—The same experiment was repeated, but in one series (A) the clot discs nearest the top of the clotted blood were used and in the other (B) the clot discs nearest the bottom were used. This was done in order to rule out, as far as possible, inaccuracies due to the uneven distribution of the bacteria in the cylindrical clot. The results of both series are given in Table V.

TABLE V.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.	
				A	B
	hrs.		*hrs.*		
Dakin's hypochlorite.	2			15	20
M/8 calcium chloride.	1	Dakin's hypochlorite.	2	20	40
M/8 sodium "	1	" "	2	10	40
9 per cent sodium chloride (normal).	3			10	

Experiment 8.—To insure still further even distribution of the bacteria through the blood clot the following variation was instituted. A rabbit was injected in-

TABLE VI.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 sodium chloride.	2			Confluent.
M/8 calcium "	2			"
Dakin's 0.5 per cent hypochlorite.	2			"
M/8 sodium chloride.	1	Dakin's 0.5 per cent hypochlorite.	2	"
M/8 calcium "	1	Dakin's 0.5 per cent hypochlorite.	2	"

travenously with 15 cc. of a 24 hour bouillon culture of *Staphylococcus aureus* and 1 minute later bled from the heart. The blood was allowed to clot in the usual manner and discs were prepared as described above. The discs were placed in solutions, taken out, ground as described above, inoculated into agar tubes, and later poured in plates, as in previous experiments. The results are shown in Table VI.

Experiments 9 and 10.—The same experiment was repeated on two later occasions with the results shown in Tables VII and VIII.

TABLE VII.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 sodium chloride.	2			50
M/8 calcium "	2			200
Dakin's 0.5 per cent hypochlorite.	2			50
M/8 sodium chloride.	1	Dakin's 0.5 per cent hypochlorite.	2	100
M/8 calcium "	1	Dakin's 0.5 per cent hypochlorite.	2	50

TABLE VIII.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
M/8 sodium chloride.	2			Confluent.
Chloramine-T 2 per cent.	2			"
Dakin's 0.5 per cent hypochlorite.	2			"
Eusol (0.5 per cent hypochlorite).	2			"
M/8 sodium chloride.	1	Chloramine-T 2 per cent.	2	"
M/8 " "	1	Dakin's 0.5 per cent hypochlorite.	2	"
M/8 " "	1	Eusol (0.5 per cent hypochlorite).	2	"

Experiment 11.—Equal sized discs of blood clot, through which *Staphylococcus aureus* had been equally distributed by the *intra vitam*, intravenous injection of the organisms in the usual manner, were placed first in a trypsin solution and later in Dakin's solution to determine whether the trypsin, by partial disruption of the

clot, rendered it more permeable to the antiseptic solution. A 1 per cent solution of commercial trypsin and Dakin's solution with a hypochlorite concentration of 0.5 per cent were used. The results of this experiment are given in Table IX.

TABLE IX.

Solution 1.	Time of contact.	Solution 2.	Time of contact.	No. of colonies.
	hrs.		*hrs.*	
Trypsin 1 per cent.	2	Dakin's solution.	2	Confluent.
" 1 " "	24	" "	2	"
Saline solution.	2			"

Inasmuch as blood clot had been found so resistant to hypochlorite solutions which readily dissolve necrotic tissue, pus cells, and other organic matter, a series of experiments was performed to determine, if possible, the resistant constituent of the clot. The results are summarized in the description of Experiment 12.

Experiment 12.—(*a*) Plasma clot was prepared by centrifuging at high speed freshly drawn rabbit blood. The resulting supernatant, clear fluid quickly coagulated. This clot, without corpuscles, is readily dissolved by Dakin's hypochlorite solution having a sodium hypochlorite concentration of 0.5 per cent. 2 per cent chloramine-T solution is without such action.

(*b*) Red blood corpuscles, prepared by defibrinating freshly drawn rabbit blood and adding sufficient saline solution to make a 5 per cent suspension of cells, are readily dissolved by the hypochlorite solution. The chloramine-T solution merely laked the corpuscles without exhibiting solvent action.

(*c*) Pus cells, prepared from human exudate and from dogs in response to the irritant action of aleuronat, are dissolved by the hypochlorite but not by the chloramine-T solution.

(*d*) It seemed probable that the fibrin of blood clot was more resistant than the fibrin of the plasma clot because the fibrin is held together in closer mechanical mass by the blood cells than in the more loosely formed plasma.[5]

[5] Experiments were undertaken but proved inconclusive and were interrupted by Dr. Taylor's death.

SUMMARY.

This work demonstrates that the chlorinated antiseptics have no power to penetrate blood clots and destroy bacteria therein contained. Correspondingly, blood clots may protect virulent bacteria for a long period of time and the organisms properly planted will be able to proliferate in a normal manner.[6,7]

[6] It seems probable that the fibrin of the blood clot is the resistant substance as plasma and red and white cells are easily dissolved by these antiseptics.

[7] The work on this paper up to this point had been written up by Dr. Taylor before his death. No attempt has been made to discuss his clear-cut and conclusive experiments. The summary given here is taken from the quarterly report rendered to the Director of The Rockefeller Institute.

THE TROPISTIC ACTION OF BLOOD VESSELS ON THE MIGRATION OF CHROMATOPHORES.

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Loeb[1] has shown that the chromatophores appearing in the yolk sac early in the development of *Fundulus heteroclitus* are attracted to the blood vessels when there is an active circulation. Thus the little red and black cells, each with many ramifying processes, begin to migrate from their scattered positions in the yolk sac to the blood vessels of this organ only after the active heart is propelling the blood through the vessels. If potassium chloride, in quantities sufficient to stop the heart beat and circulation, is added to the water containing the developing embryos, the chromatophores develop normally, but until the 4th day, at least, there is no migration comparable with that observed in controls where by this time the pigment cells are definitely approaching the vessels. Loeb suggests that there is probably something present in the circulating blood which is responsible for this tropistic phenomenon.

The experiments recorded below were devised for the purpose of arriving at a satisfactory explanation of the phenomenon, if possible.[2]

Experiment 1.—60 eggs of *Fundulus heteroclitus*, artificially fertilized, were kept in sea water. After 4 days incubation black and red chromatophores, with widely spread out processes were scattered over the yolk sac. By this time the hearts had begun to beat and the blood was circulating through the thin walled blood vessels which formed a network over the surface of the yolk sac. None of the chromatophores showed any relation to the vessels at this time. On the 6th day of incubation, however, the chromatophores had approached and embraced the blood vessels of the yolk sac so that the location of the vessels could be accurately determined by the coloring of their periphery caused by the closely

[1] Loeb, J., *J. Morphol.*, 1893, viii, 161; On the heredity of the marking in fish embryos, Biological lectures, Boston, 1899, 227.

[2] These experiments were suggested by Dr. Jacques Loeb.

approximated black and not quite so closely approximated red chromatophores. Twenty of the eggs were allowed to remain in the sea water, twenty transferred to $\frac{M}{8}$ potassium chloride in sea water, and twenty to $\frac{M}{16}$ potassium chloride in sea water. The results are summarized in Table I.

TABLE I.

Length of time after fertilization.	Sea water.		M/16 KCl		Transferred from M/16 KCl to sea water.*		Transferred from M/8 KCl to sea water.*	
	No. with active circulation. (Chromatophores on vessels.)	No. with inactive circulation. (Chromatophores spread.)	No. with active circulation. (Chromatophores on vessels.)	No. with inactive circulation. (Chromatophores spread.)	No. with active circulation. (Chromatophores on vessels.)	No. with inactive circulation. (Chromatophores spread.)	No. with active circulation. (Chromatophores on vessels.)	No with inactive circulation. (Chromatophores spread.)
days	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
6	100	0	100	0				
8	100	0	100	0				
9	100	0	45	55				
10	100	0	50	50	60	40	0	100
11	100	0	10	90	40	60	0	100
16	All hatched swimming.		0	100	55	45	10	90
17			0	100	30	70	10	90
18			0	100	45	55	10	90
19			0	100	33	67	20	80
20			0	100	33	67	10	90
25			All dead.		55	45	0	100
26					45	55	0	100
27					70	30	0	100
41					100	0	All dead.	
46					0	100		

* Left in $\frac{M}{8}$ potassium chloride and $\frac{M}{16}$ potassium chloride for 4 days (from 6th to 10th day of incubation period) and then placed in sea water.

The chromatophores of all the embryos developing in sea water alone remained in close contact with the blood vessels until the time of hatching (16th day after fertilization). All hatched, and the fish were apparently normal.

In those left in $\frac{M}{16}$ potassium chloride the hearts and circulations gradually became inactive and as this occurred the chromatophores again became spread out on the surface of the yolk sac. None of these embryos ever hatched. Ten of the latter after 4 days in the $\frac{M}{16}$ potassium chloride (from the 6th to the 10th day of incubation)

were again returned to sea water without excess potassium. At that time 60 per cent still had active hearts and circulations, and the chromatophores were closely embracing the vessels. In 40 per cent the hearts had stopped, the blood was not circulating, and the chromatophores were spread out over the surface of the yolk sac. At the observations made after this time there were at various times different percentages of embryos with active hearts and circulations but always those in which the blood was flowing through the vessels also had the chromatophores in close contact with the blood vessels. Contrasting with this the chromatophores were always spread out over the surface of the yolk sac when the circulation was inactive. On the 41st day of incubation all had active circulation and chromatophores closely approximating the blood vessels. Thus on the 46th day of incubation, which was as far as the observations were continued, 100 per cent showed spread out chromatophores, and in none of these was there an active circulation.

In those left in $\frac{M}{8}$ potassium chloride and later transferred to sea water without excess potassium chloride a few again developed active circulations and in these the chromatophores again approximated and embraced the vessels in the characteristic manner. In those with inactive circulation, however, the chromatophores remained scattered and showed no relation to the blood vessels. When the circulation again stopped, in those that had begun a second time, the chromatophores again became spread out in the same manner.

A few confirmatory observations of interest may be briefly considered here.

1. When the circulation had only been inactive for 24 hours or less as in the column headed "Transferred from $\frac{M}{16}$ KCl to sea water," on the 11th day of incubation, it could be seen that the embryos in which the circulation had last stopped showed a much less pronounced wandering and spreading over of the chromatophores than those in which it had not started again.

2. The result was similar when the circulation had started but 24 hours or less before observation, in the column headed "Transferred from $\frac{M}{16}$ KCl to sea water," on the 18th day of incubation. Thus two of the three embryos in this category showed only partial approximation of the chromatophores to the vessels and not the complete stage noted in the remainder.

3. Sometimes there was an embryo present whose heart gave an occasional spasmodic beat, in the column headed "$\frac{M}{16}$ KCl," on the 11th day of incubation, and in these the chromatophores had not migrated far from the blood vessels and became now partially spread out. This is what would be expected if, as seems probable, this was the last in which the circulation had stopped.

4. In the embryos with beating heart but inactive circulation, in the column headed "Transferred from $\frac{M}{8}$ KCl to sea water," on the 16th day of incubation, there is usually a clump of chromatophores closely approximating the base of the beating heart where it is fixed to the surface of the yolk sac. It seems suggestive that chromatophores collect about the beating heart after they have left the vessels through which the blood has ceased to flow.

5. In other embryos with slow circulation following the removal of the stopped heart from $\frac{M}{8}$ potassium chloride to sea water, we find all stages in the restoration of the circulation and where this is rapid the chromatophores closely approximate the vessels, where slow they form but a loose meshwork about the vessels.

Experiment 2.—This experiment was in every way comparable with Experiment 1.

Experiment 3.—Eggs of the same teleost were allowed to develop normally until the 6th day when they were placed in flasks containing sea water to which had been added a few drops of a dilute solution of sodium cyanide (0.1 per cent). Eggs placed in 50 cc. of sea water to which two drops of the sodium cyanide solution had been added, when removed, at the end of 9 days, still had active circulations. The chromatophores were in about the same state as at the time when the eggs were first placed in the cyanide solution; *i.e.*, most were approximated and had embraced the vessels, but a few, especially those of the red variety, were still partially spread out over the surface of the yolk sac. At this time the controls, which had been kept in sea water without cyanide, had hatched. Those placed in the cyanide never hatched. When kept for 9 days in 50 cc. of sea water plus two drops of sodium cyanide the circulations were inactive but the hearts gave occasional spasmodic beats or beat steadily. The chromatophores had not spread out as they did when the circulation was stopped before the heart in Experiments 1 and 2. After removal from the cyanide to sea water or potassium chloride solutions the chromatophores behave exactly as they did under similar conditions in Experiments 1 and 2. The cyanide by lowering the rate of oxidation may have interfered with the motility of the chromatophores.

Experiments with alcohol were next tried.

Eggs fertilized 4 days previously were placed in sea water to which absolute ethyl alcohol had been added in sufficient quantity to make solutions of 2, 3, 4, and 5 per cent respectively. Thirty eggs were placed in each solution. After 18 hours ten eggs were removed from each solution and placed in sea water. The same procedure was repeated after 24 and again after 30 hours and each lot of eggs, separately kept, was observed at frequent intervals. Table II gives the results.

TABLE II.

Day of incubation.	2 per cent.			3 per cent.			4 per cent.			5 per cent.			Control.
	18 hrs.	24 hrs.	30 hrs.	18 hrs.	24 hrs.	30 hrs.	18 hrs.	24 hrs.	30 hrs.	18 hrs.	24 hrs.	30 hrs.	
5	++	++	++	++	++	++	++	+	+	0	0	0	++
6	++	++	++	++	++	++	++	+	+	+	0	0	++
10	++	++	++	++	++	++	++	++	+	++	+	0	++
20	H.	H.	H.	H.	H.	H.	H.	H.	H.	H.	+	D.	H.
30											+		

++, hearts and circulations active.
+, hearts but not circulations active.
0, neither hearts nor circulations active.
H., hatched.
D., dead.

In Table II majorities are indicated as the results were too irregular to record *in toto* without too much complexity. However, the results in each instance are similar to those encountered in previous experiments in every particular. Where the circulation is active (++), the chromatophores closely approximate the blood vessels and tightly embrace them. The embracing process is less complete the shorter the time since the circulation has again commenced; *i.e.*, 5 per cent, 10th day of incubation. When the heart is active but the circulation is at a standstill (+), the chromatophores are widely spread out over the yolk sac; *i.e.*, 5 per cent, 6th day of incubation. This is also the case when neither the heart nor the circulation is active (0); *i.e.*, 5 per cent, 5th day of incubation. There was considerable mortality among the embryos treated with alcohol, and in certain instances the circulations in some of the embryos were inactive while others were active. The tendency is indicated by the graphic record of the majority. When variations occurred there was always the consistent relation between the circulation and the chromatophores.

DISCUSSION.

The distribution and arrangement of the chromatophores of the yolk sac of embryonic *Fundulus heteroclitus* seem to be determined by their relation to the most abundant supply of oxygen or to the lower hydrogen ion concentration. Thus, before there is any circulation they are spread out widely on the surface of the yolk sac without regard to the blood vessels. As soon as the circulation starts, the chromatophores approach the capillary, creeping longitudinally along the surface of, coupling up with those adjacent, and finally, forming an almost continuous covering of the vessels of the yolk sac. It may be presumed that at this time the tension of oxygen is higher and the hydrogen ion concentration lower in the circulating blood than in the tissue cells. If the circulation is stopped by any means, potassium chloride, sodium cyanide, ethyl alcohol, even with a beating heart, the chromatophores tend to spread out once more over the surface of the yolk sac in such a way that the greatest possible surface area is approximated to the sea water surrounding the embryo. With a beating heart, however, a clump of chromatophores is often found about the base of this organ, for reasons not entirely clear. In the experiments recorded above all stages in the development of a circulation and the cessation of one once begun have been encountered and it may be safely stated that there seems to be a direct relation between an active circulation and a close approximation and envelopment of the vessels by the chromatophores. A substance which, like sodium cyanide, inhibits oxidation also inhibits further migrations of the chromatophores, probably by paralyzing the latter; the tropistic reactions of the chromatophores are resumed when the poison (sodium cyanide) is removed and the eggs are transferred to a proper medium (sea water). When the eggs die the chromatophores shrivel up and appear as small dark spots against the opaque background of the egg. The red chromatophores respond much more slowly to an adequate stimulus than the black ones.

CONCLUSIONS.

1. The migrations of the chromatophores of the yolk sac of *Fundulus heteroclitus* seem to be chemotropic in character.

2. The determining factor seems to be either a high tension of oxygen or a comparatively low concentration of hydrogen ions.